(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 218 391 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
11.04.2007 Bulletin 2007/15

(21) Application number: **00970403.2**

(22) Date of filing: **05.10.2000**

(51) Int Cl.:
C07H 19/06 *(2006.01)* ***C07H 19/10*** *(2006.01)*
C07H 19/16 *(2006.01)* ***C07H 19/20*** *(2006.01)*
C07H 21/00 *(2006.01)* ***C12Q 1/68*** *(2006.01)*

(86) International application number:
PCT/SE2000/001929

(87) International publication number:
WO 2001/025247 (12.04.2001 Gazette 2001/15)

(54) COMPOUNDS FOR PROTECTING HYDROXYLS AND METHODS FOR THEIR USE

VERBINDUNGEN ZUM SCHUTZ VON HYDROXYLGRUPPEN UND IHRE ANWENDUNG

COMPOSES DE PROTECTION DES HYDROXYLES ET PROCEDES D'UTILISATION

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **05.10.1999 US 412171**

(43) Date of publication of application:
03.07.2002 Bulletin 2002/27

(73) Proprietor: **Helicos Biosciences Corporation**
Cambridge, MA 02139 (US)

(72) Inventor: **KWIATKOWSKI, Marek**
S-756 52 Uppsala (SE)

(74) Representative: **Fagerlin, Heléne et al**
Albihns Stockholm AB
Box 5581
114 85 Stockholm (SE)

(56) References cited:
WO-A1-91/00868
WO-A1-95/31434
WO-A1-96/23807
WO-A1-98/08857
WO-A1-98/39481

- **TETRAHEDRON LETTERS, Volume 32, No. 26, 1991, E. Bonfils et al, "Solid Phase synthesis of 5',3'-Bifunctional Oligodeoxyribonucleotides Bearing a Masked Thiol Group at the 3'-End" pages 3053 - 3056, XP002901524**
- **BRESLOW; CHUNG: 'Strong binding of ditopic substrates by a doubly linked occlusive C1 "clamshell" as distinguihsed from an aversive C2 "loveseat" cyclodextrin' JOURNAL OF THE AMERICAN CHEMICAL SOCIETY vol. 112, 1990, pages 9659 - 9660**
- **POLNIASZEK; STEVENS: 'Preparation of potential intermediates for the synthesis of yohimbine and reserpine.' JOURNAL OF THE ORGANIC CHEMISTRY vol. 51, 1986, pages 3023 - 3027**
- **ISAKA ET AL: 'Synthesis of esters of ampicillin' YAMANOUCHI SEIYAKU KENKYU HOKOKU vol. 1974, no. 2, 1974, pages 95 - 108, XP009054423**
- **TSUDA ET AL: 'Total synthesis of homoerythrinan alkaloids, schelhammericine and *-pischelhammericine' CHEM. PHARM. BULL. vol. 44, no. 3, 1996, pages 500 - 508**

EP 1 218 391 B1

Description

Field of the Invention

[0001] The invention relates to biological chemistry in general. In particular, the invention relates to protecting hydroxyls in organic molecules.

Background of the Invention

[0002] Temporary protection or blocking of chemically reactive functions in biological compounds is an important tool in the field of biological chemistry. To this end, researchers have developed a number of protecting groups. The vast majority of the known protecting groups, however, are acid or base labile and while there are also protecting groups that are labile under neutral conditions, most of these protecting groups are also somewhat acid and base labile. Greene, TW, "Protective Groups in Organic Synthesis", publishers *Wiley-Interscience* (1981). Furthermore, many protecting groups suffer additional synthesis, side-reaction, and/or solubility problems. For example, only a few protecting groups applied as a part of a linking system between the solid phase and the oligonucleotide can withstand all the rigors of oligonucleotide synthesis and deprotection thereby facilitating the final purification of oligonucleotides free of truncated or depurinated fragments. See "Solid Phase Synthesis," Kwaitkowski et al., PCT International Publication WO 98/08857 (1996). Selective post-synthetic derivatization of oligonucleotides also requires selectively cleavable protecting groups. See, e.g., Kahl & Greenberg, "Introducing Structural Diversity in Oligonucleotides via Photolabile, Convertible C5-substituted Nucleotides," *J. Am. Chem. Soc.,* 121(4), 597-604 (1999).

[0003] Protecting groups also should be removable. Ideally, the protecting group is removable under mild conditions, for example, without disturbing interactions between biomolecules. These types of protecting groups may be useful for deprotecting oligonucleotides without disturbing interactions between oligo/polynucleotide strands. For example, International Publication WO 96/23807 entitled "Novel Chain Terminators, The Use Thereof for Nucleic Acid Sequencing and Synthesis and a Method of their Preparation" discloses methods that use nucleotides that are reversibly blocked at the 3' hydroxyl group. These reversibly blocked nucleotides can be used in sequencing methods where, unlike the well-known Sanger sequencing method that utilizes terminating dideoxynucleotides, the temporarily 3'-OH-protected intermediates can be converted into nucleotides having a free 3'-OH that may be further extended.

[0004] One such sequencing method that uses reversibly blocked nucleotides is known as Sequencing by Synthesis (SBS). SBS determines the DNA sequence by incorporating nucleotides and detecting the sequence one base at a time. To effectively sequence long stretches of a nucleic acid using SBS, it is advantageous to be able to perform multiple iterations of the single nucleotide incorporation. Accordingly, SBS-based methods require 3'-OH protecting groups that are removable under conditions that do not distrupt the primer and target DNA interactions. As such, there exists a need for nucleotide triphosphates that are reversibly blocked at the 3' position and which are also effective substrates for DNA polymerases

[0005] Tetrahedon Letters, Vol 32, No 26, pp 3053-3056 describes bifunctional oligodeoxyribonucleotides bearing a masked thiol group at the 3'-end. The thiol group is not accepted by a polymerase and thus no sequencing reaction can be made using primers modified by this method. The present methodology, in contrary, is the only one that provides the necessary 3' OH, and makes use of a linker that is stable under strongly basic conditions that are used during oligonucleotide deprotection.

Summary of the Invention

[0006] In one aspect, the invention provides a hydrocarbyldithiomethyl-modified compound of the Formula:

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

or a salt thereof, wherein

R^1 is chosen from modified or unmodified amino acids, peptides, proteins, carbohydrates, sterols, steroids, ribonucleosides ribonucleotides, base and/or sugar modified ribonucleosides, base- and/or sugar-modified ribonucleotides, deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides; and

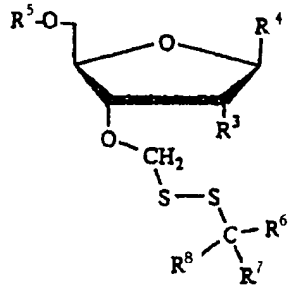
R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups. R^1 has at least one hydroxyl group, which after modification is in an ether linkage. In one embodiment, R^2 further

includes a labeling group. The labeling group can be any type of labeling group including fluorescent labeling groups, which can be selected from the group consisting of Bodipy, Dansyl™, fluorescein, rhodamin, Texas red™, Cy 2™, Cy 4™, and Cy 6™.

[0007] R^1 can have more than one hydroxyl group and more than one of the hydroxyl groups can be modified with a dithiomethyl moiety. For nucleotide embodiments, the dithiomethyl modification can be at the 2' and/or, 3', and/or 5' hydroxyl positions of the R^1-O.

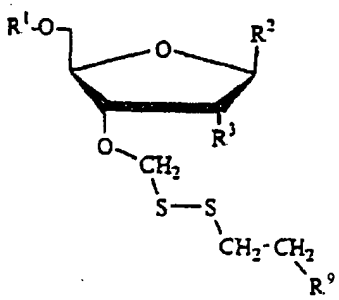
[0008] In another embodiment, R^2 includes a function that modifies the electron density of the dithio function, thereby modifying the stability of the dithiol. Such a function may be provided by a chemical group containing elements selected from the group consisting of oxygen, nitrogen, sulfur, and silicon.

[0009] In another aspect, the invention provides a dithiomethyl-modified compound of the Formula:



or a salt thereof, wherein R^5 is H, a protecting group, phosphate, diphosphate, triphosphate, or residue of a nucleic acid, R^2 is a nucleobase, R^3 is H, OH, or a protected form of OH; and R^6, R^7 and R^8 are together or separately H, hydrocarbyl, or a residue of a solid support. Suitable hydrocarbyls for R^6, R^7 and R^8 include methyl, ethyl, isopropyl, and t-butyl. In one embodiment, R^6, R^7 and R^8 together or separately further include a labeling group and/or an electron donating function or electron density modifying function. The electron density modifying function can be a heteroatom selected from the group consisting of oxygen, nitrogen, sulfur, and silicon.

[0010] In another aspect, the invention provides a compound of the Formula:



or a salt thereof, wherein R^1 is H, a protecting group, a phosphate, diphosphate, or a triphosphate, or a residue of a nucleic acid, R^2 is nucleobase, R^3 is H or OH, or a protected form of OH, and R^9 is H or any organic radical having a carbon atom directly attached to the remainder of the molecule, chosen from, saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups. In one embodiment, R^9 is modified with a labeling group. In other embodiments, R^9 includes a derivatizable function, or R^9 includes nitrogen, or R^9 is covalently linked to a solid support.

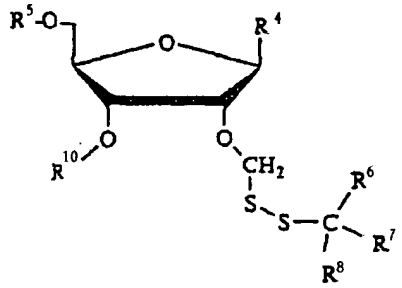
[0011] In another aspect, the invention provides a method for modifying a nucleoside including the steps of a) contacting a nucleoside having at least one hallogenomethyl-modified hydroxyl group with an thiosulfonate compound thereby forming a thiosulfonated nucleoside; and b) contacting the thiosulfonated nucleoside with a hydrocarbylthiol compound thereby forming a dithiomethyl-modified nucleoside. Useful thiosulfonate compounds include alkylthiosulfonate and arylthiosulfonate.

[0012] In one embodiment, the method includes the step of labeling the dithiomethyl-modified nucleoside.

[0013] In another aspect, the invention provides a method for sequencing a nucleic acid including the steps of: a) contacting a target nucleic acid with a primer wherein at least a portion of the primer is complementary to a portion of the target nucleic acid; b) incorporating a dithiomethyl-modified nucleotide into the primer, and c) detecting incorporation of the dithiomethyl-modified nucleotide, wherein the dithiomethyl-modified nucleotide is complementary to the target nucleic acid at the dithiomethyl-modified nucleotide's site of incorporation. In one embodiment, the incorporating step is catalyzed by a DNA polymerase. Useful sequencing methods that may use the method disclosed above include minisequencing and sequencing by synthesis whether performed in isolation or performed as a sequencing array

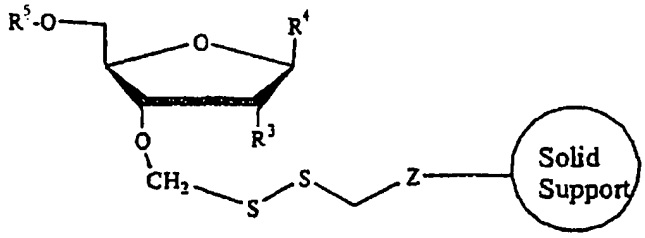
[0014] In another aspect, the invention provides a method for sequencing a nucleic acid including the steps of: a) contacting a target nucleic acid with a primer wherein at least a portion of the primer is complementary to a portion of the target nucleic acid; b) incorporating a first dithiomethyl-modified nucleotide into the primer; c) detecting the incorporation of the first dithiomethyl-modified nucleotide; d) removing the dithiomethyl group from the first incorporated dithiomethyl-modified nucleotide to form a first elongated primer having a free hydroxyl group; e) incorporating a second dithiomethyl-modified nucleotide into the first elongated primer; and f) detecting the second dithiomethyl-modified nucleotide, wherein the first dithiomethyl-modified nucleotide and the second dithiomethyl-modified nucleotide are complementary to the target nucleic acid at each nucleotide's site of incorporation. Following the sequencing method steps once will identify the sequence of one nucleobase of the target nucleic acid. Repeating the steps can facilitate identifying the sequence of more than one nucleobase of the target nucleic acid. The conditions of the sequencing method should be such that the primer anneals or hybridizes to the target nucleic acid in a sequence specific manner. In some embodiments the detecting steps are performed before removing the dithiomethyl group whereas in other embodiments the detecting the incorporation steps are performed after removing the dithiomethyl group. In some embodiments, the method is optimized for implementing the method in a sequencing array.

[0015] In another aspect, the invention provides a compound of the Formula:



wherein R^5 is a H, a protecting group, a phosphate, diphosphate, or a triphosphate, or a residue of a nucleic acid; R^4 is a nucleobase; R^6, R^7 and R^8 are together or separately H or any organic radical having a carbon atom directly attached to the remainder of the molecule, chosen from, saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups and R^{10} is H, H-phosphonate or phosphoramidite.

[0016] In another aspect, the invention provides an oligonucleotide synthesis support of the formula:



wherein R^5 is H, phosphate, diphosphate, triphosphate, or a protecting group, R^4 is a nucleobase, R^3 is H, OH, or a protected form of OH, and Z is a group effective for covalent attachment to a solid support, the solid support being

effective for securing an oligonucleotide during oligonucleotide synthesis. In some embodiments, Z is selected from the group consisting of amino, amido, ester, and ether.

[0017] In another aspect, the invention provides a method for synthesizing an oligonucleotide including the steps of: a) providing a 5' protected first nucleoside secured to a solid support through a linker; b) deprotecting the first nucleoside at its 5' position; c) covalently bonding another 5' protected nucleoside to the first nucleotide at the 5' position of the first nucleoside; d) deprotecting the another nucleoside at its 5' position; and e) repeating steps c) and d) for incorporating additional protected nucleosides. For this aspect of the invention, the linker secures the first nucleotide to the solid support via a dithiomethyl bond. This synthesizing method can be optimized for manufacturing oligonucleotide arrays.

[0018] In some embodiments, the oligonucleotide synthesis method is effective for inverting the oligonucleotide thereby forming an oligonucleotide having a free 3' hydroxyl and being secured to a solid support via another position.

[0019] In another aspect, the invention provides a method for synthesizing an oligoribonucleotide including the steps of: a) providing a first protected ribonucleoside covalently linked to a solid support; b) covalently linking at least one dithiomethyl-modified ribonucleoside to the first ribonucleoside forming an oligoribonucleotide; c) partially de-protecting the oligoribonucleotide under acidic or basic conditions; and d) contacting the oligoribonucleotide with a reducing agent under neutral conditions thereby completely de-protecting the oligoribonucleotide, wherein the dithiomethyl-modified ribonucleoside includes a dithiomethyl group bound at the 2' position of the dithiomethyl-modified ribonucleoside. Such a method is effective for preventing cleavage or migration of internucleotide phosphate bonds during deprotection and is also effective for inverting the oligoribonucleotide thereby forming a solid phase bound oligonucleotide having a free 3' hydroxyl. In some embodiments, the pH is neutral and can be about 7 or range from about 5 to about 9. In other embodiments, the first protected ribonucleoside is secured or covalently linked to the solid support via a dithiomethyl bond.

[0020] In another aspect, the invention provides a method for sequencing a nucleic acid including the steps of a) providing a primer array including a plurality of sequencing primers; b) contacting a target nucleic acid with the primer array thereby forming target-primer complexes between complementary portions of the sequencing primers and the target nucleic acid; c) incorporating a first dithiomethyl-modified nucleotide into at least one sequencing primer portion of the target-primer complexes, the first dithiomethyl-modified nucleotide being complementary to the target nucleic acid; and d) detecting the incorporation of the first dithiomethyl-modified nucleotide, wherein the first dithiomethyl-modified nucleotide is complementary to the target sequence at the first dithiomethyl-modified nucleotide's site af incorporation. In one embodiment, the method further includes the steps of: e) removing the dithiomethyl group from the first incorporated dithiomethyl-modified nucleotide to form a first elongated target-primer complex having a free 3' hydroxyl group; f) incorporating a second dithiomethyl-modified nucleotide into the first elongated target-primer complex; and g) detecting the second dithiomethyl-modified nucleotide, wherein the second dithiomethyl-modified nucleotide is complementary to the target sequence at the second dithiomethyl-modified nucleotide's site of incorporation. As with other methods described herein, the detecting step can be performed before or after removing a dithiomethyl moiety. This sequencing method is effective for producing a plurality of nucleotide sequences wherein the nucleotide sequences correspond to overlapping nucleotide sequences of the target nucleic acid.

[0021] In another aspect, the invention provides a method for sequencing a nucleic acid including the steps of: a) providing a target nucleic acid array including a plurality of target nucleic acids; b) contacting a sequencing primer with the target nucleic acids thereby forming target-primer complexes between complementary portions of the sequencing primers and the target nucleic acids; c) incorporating a first dithiomethyl-modified nucleotide into at least one sequencing primer portion of the target-primer complexes, the first dithiomethyl-modified nucleotide being complementary to the target nucleic acid; and d) detecting the incorporation of the first dithiomethyl-modified nucleotide, wherein the first dithiomethyl-modified nucleotide is complementary to the target sequence at the first dithiomethyl-modified nucleotide's site of incorporation. As with other methods described herein, the detecting step can be performed before or after removing a dithiomethyl moiety. This sequencing method is effective for producing a plurality of nucleotide sequences wherein the nucleotide sequences correspond to overlapping nucleotide sequences of the target nucleic acid.

[0022] In another aspect, the invention provides a method for synthesizing an oligonucleotide that includes the steps of: a) providing a 5' protected first nucleoside covalently bonded to a solid support through a dithiomethyl containing linker; b) deprotecting the first nucleoside at its 5' position; c) covalently bonding another 5' protected nucleoside to the first nucleotide at the 5' position of the first nucleoside; d) deprotecting the another nucleoside at its 5' position; e) optionally repeating steps c) and d) for adding additional protected nucleosides thereby producing an oligonucleotide; f) optionally selectively cleaving a protecting group from the oligonucleotide thereby forming a partially deprotected oligonucleotide; g) selectively cleaving the dithiomethyl containing linker, and h) isolating the partially deprotected oligonucleotide. In one embodiment, the method further includes the step of modifying the 3' terminus with a reactive or detectable moiety. In another embodiment, at least one of the 5' protected nucleosides contains a dithiomethyl moiety.

[0023] Advantages of the invention include introducing temporary or reversible mutations in proteins, facilitating continuous sequencing methods, blocking reactive species during chemical syntheses, masking chemical groups for manufacturing purposes, using hydroxyl groups to introduce labeling groups into organic molecules, and other similar uses. It is to be understood that particular embodiments of the invention described herein may be interchanged with other

embodiments of the invention.

[0024] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0025] Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Detailed Description

[0026] The present invention relates to dithiomethyl-modified compounds having modified and/or protected hydroxyl groups, methods for manufacturing such compounds, and methods for their use. The general formula for the hydroxyl-modifying moiety contains a dithiol. The dithiol is designed so that modified hydroxyls can be deprotected under neutral conditions using mild reducing agents.

[0027] A dithiomethyl-modified compound is shown in Formula 1:

$$R^1\text{-O-CH}_2\text{-S-S-}R^2 \qquad \text{(Formula 1)}$$

wherein R^1 represents any organic molecule that had at least one free hydroxyl group before undergoing the dithiomethyl modification. In Formula 1, "O" is the oxygen atom of the hydroxyl group, which is now protected in an ether linkage. For example, before modification, R^1 can be a modified or unmodified amino acid or analog thereof, oligonucleotide, peptide, protein, carbohydrate, deoxyribonucleoside, deoxyribonucleotide, ribonucleoside, ribonucleotide, base- and/or sugar-modified ribonucleoside, base- and/or sugar-modified deoxyribonucleoside, base- and/or sugar-modified nucleotide, sterol or steroid, as long as the organic molecule selected has at least one hydroxyl group capable of being dithiomethyl modified. As referred to herein, oligonucleotides refers to any nucleotide polymer including polymers of deoxyribonucleotides, ribonucleotides, nucleotide analogs and mixtures thereof.

[0028] When R^1-O is an organic molecule having more than one free hydroxyl group, any number of the free hydroxyls may be modified with a dithiomethyl moiety. Alternatively, one or more of the additional hydroxyl groups can be modified and/or protected with other known hydroxyl modifying compounds or left unmodified. Different protecting groups may be used to protect different hydroxyl groups. Useful protecting groups, other than the dithiomethyl-based groups described herein, and methods for their use are known to those of skill in the art and include fluorenylmethyloxycarbonyl (FMOC), 4-(anisyl)diphenylmethyltrityl (MMTr), dimethoxytrityl (DMTr), monamethoxytrityl, trityl (Tr), benzoyl (Bz), isobutyryl (ib), pixyl (pi), ter-butyl-dimethylsilyl (TBMS), and 1-(2-fluorophenyl)-4-methoxypiperidin 4-yl (FPMP). See, e.g., Greene, TW, "Protective Groups in Organic Synthesis", publishers *Wiley-Interscience* (1981); Beaucage & Iyer, "Advances in the Synthesis of Oligonucleotides by the Phosphoramidite Approach," *Tetrahedron,* 48(12):2223-2311 (1992); Beaucage & Iyer, "The Synthesis of Specific Ribonucleotides and Unrelated Phosphorylated Biomolecules by the Phosphoramidite Method," *Tetrahedron,* 49(46):10441-10488 (1993); and Scaringe et al., "Novel RNA Synthesis Method Using 5'-O-silyl-2'-O-orthoester Protecting Groups," *J. Am. Chem. Soc.,* 120:11820-21 (1998). The choice of protective group can be dictated by the type of organic molecule to be protected and the methods employed. Therefore, different organic molecules such as peptides, oligonucleotides, carbohydrates, and steroids may each use different protective goups. A hydroxyl with a known protecting group or a dithiomethyl moiety attached to it can be referred to as a protected form of the hydroxyl.

[0029] In Formula 1, R^2 represents a hydrocarbyl group. As used herein, hydrocarbyl groups include any organic radical having a carbon atom directly attached to the remainder of the molecule, e.g., saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups. The hydrocarbyl group may be covalently linked to a solid support (described below), labeling group or another organic molecule.

[0030] Suitable hydrocarbons include alkyls (e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, and heptadecyl); alkoxy; alkenyl; C_{3-8} alkenyloxy; alkynyl; alkynyloxy; C_{3-20} cycloalkyl (e.g. cyclopropyl, cyclobutyl or cyclopentyl) in which the cycloalkyl may be substituted by one or more hydrocarbyls or heteroatoms; C_{3-8} cycloalkoxy (e.g. cyclopentoxy); C_{4-8} cycloalkenyloxy (e.g. cyclopenten-3-yloxy); aryl (e.g. phenyl) or aralkyl (e.g. benzyl) in which the aryl may be substituted with one or more C_{1-4} alkyl, halogen, hydroxy, C_{1-4} alkoxy, amino or nitro; aryloxy (e.g. phenoxy); arylalkoxy (e.g., benzyloxy) in which the aryl may be substituted with one or more C_{1-4} alkyl, halogen, hydroxy, C_{1-4} alkoxy, amino or nitro; C_{1-6} hydroxyalkyl (e.g. hydroxyethyl); and C_{1-6} alkoxyalkyl (e.g. methoxyethyl). In addition, all iso, sec and tert isomers of the aliphatic hydrocarbons are included such as isopropyl and t-butyl.

[0031] Substituted hydrocarbon groups are hydrocarbons containing non-hydrocarbon substituents. Suitable substit-

uents include oxygen, nitrogen, sulfur, phosphorous, halogens (e.g., bromine, chlorine, iodine, and fluorine), hydroxy, carbalkoxy (especially lower carbalkoxy) and alkoxy (especially lower alkoxy), the term, "lower" denoting groups containing 7 or less carbon atoms.

[0032] Other functional modifying groups capable of moderating the reactivity or lability of the disulfide bond or facilitate synthesizing compounds of Formula 1 can be incorporated into R^2. Useful functional modifying groups are known and include heteroatoms such as oxygen, nitrogen, sulfur, phosphorous, and halogens. Functional modifying groups also include heterogroups such as amino, nitro, and cyano. These groups may function as an electron withdrawing or donating groups. Skilled artisans know whether electron withdrawing or donating groups would be appropriate.

[0033] R^2 may further include a labeling group. Useful labeling groups are known to those of ordinary skill in the art and include radioactively labeled groups, luminescent groups, electroluminescent groups, fluorescent groups, and groups that absorb visible or infrared light Examples of useful fluorescent labels include Bodipy™, Dansyl™, fluorescein, rhodamin, Texas red™, Cy 2™, Cy 4™, and Cy 6™. Additional useful labels can be found in the "Handbook of Fluorescent probes and Research Chemicals," by Richard P. Haugland and "Nonisotopic DNA Probe Techniques," Ed. Larry J. Kricka (Academic Press, Inc. 1992), dithiomethyl-modified compounds can be created from available compounds using the following illustrative method. A free hydroxyl on a hydroxyl-containing molecule is modified to form a methylthiomethyl ether. The methylthiomethyl ether can be formed by reacting the hydroxyl with a mixture of acetic anhydride, acetic acid and dimethyl sulfoxide (DMSO). See Hovinen et al., "Synthesis of 3'-O-(ω-Aminoalkoxymethyl)thymidine 5'-Triphosphates, Terminators of DNA synthesis that Enable 3'-Labeling," *J. Chem. Soc. Perkin Trans. I,* pp. 211 -217 (1994). It is to be understood that, if the molecule to be modified contains more than one hydroxyl group, it may be necessary to first protect or block one or more hydroxyl groups that are not to be dithiomethyl-modified.

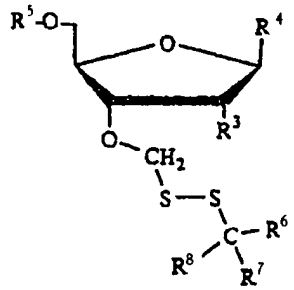
[0034] The methylthiomethyl ether-derivatized compound is then converted to a more reactive species such as a halogenatedmethyl ether. Useful halogens include bromine, chlorine, and iodine. The halogenation step can be carried out using any method including treating the methylthiomethyl ether with N-bromosuccinimide (NBS), or Br_2 in dry chlorethane, or $SOCl_2$, or N-iodosuccinimide (NIS).

[0035] The halogenated methyl ether compound is then converted to a hydrocarbylthiolsulfonate reagent by treating it with an alkyl hydrocarbylthiolsulfonate. See Bruice & Kenyon, "Novel Alkyl Alkanethiolsulfonate Sulfhydryl reagents, Modification of Derivatives of L,-Cysteine," *J. Protein Chem.,* 1(1):47-58 (1982) and Plettner et al., "A Combinatorial Approach to Chemical Modification of Subtilisin *Bacillus lentus," Bioorganic & Medicinal Chem. Lett.* 8, pp. 2291-96 (1998).

[0036] Contacting the hydrocarbylthiolsulfonate reagent with any unsubstituted or substituted thiol can cause displacement of the sulfonyl moiety thereby creating a dithiomethyl-modified compound. Useful thiols include branched- and straight-chain aliphatic thiols, aromatic thiols, heteroaromatic thiols, substituted aliphatic thiols, functionally modified thiols, and fluorophore labeled thiols. Functionally modified thiols include thiols substituted at a carbon atom with an atom or group capable of altering the reactivity of a dithio moiety, capable of facilitating subsequent labeling, or capable of facilitating immobilization of the modified compound. Useful examples of modifying groups include amino, amido, hydroxyl, silyl, cyano, carboxylic esters, or other carboxylic substitutions.

[0037] It may be particularly useful to use the compounds of Formula 1 when R^1 contains a nucleobase. As used herein, nucleobase includes any natural nucleobase, synthetic nucleobase, and/or analog thereof. Natural nucleobases include adenine, guanine, cytosine, thymine, uracil, xanthine, hypoxanthine, and 2-aminopurine. Synthetic nucleobases are typically chemically synthesized and are analogues of the natural nucleobases. Synthetic nucleobases are capable of interacting or hydrogen bonding with other nucleobases. Nucleobase containing compounds can include both nucleosides and nucleotides. Nucleosides and nucleotides can be modified at the 5', 3' and/or 2' hydroxyl positions. Known methods for protecting the 5', 3' and/or 2' positions may be used in conjunction with the methods described herein to modify individual hydroxyl positions.

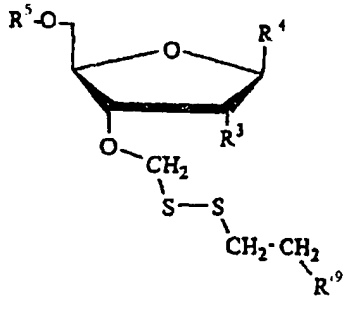
[0038] For example, the compound shown in Formula 2



(Formula 2)

or a salt thereof can be synthesized using the methods described herein. In Formula 2, R^5 is H, a protecting group, phosphate, diphosphate, triphosphate, or a residue of a nucleic acid; R^4 is a nucleobase; R^3 is H, OH, or a protected form of OH; R^6, $R^7$and R^8 are together or separately H, hydrocarbyl, or a residue of a solid support. For example, R^6, R^7 and R^8 include together or separately H, methyl, ethyl, isopropyl, t-butyl, phenyl, or benzyl.. It may be useful to include a substituted hydrocarbon having an electron density modifying group containing a heteroatom or other functional modifying group at positions R^6, R^7 or R^8. For example, R^6, R^7 or R^8 could be methyleneamine, ethyleneamine, or contain an amino group. As an optional aspect, R^6, R^7 or R^8 can be modified with a labeling group.

[0039] Another illustrative example of useful dithiomethyl-modified compounds include the compounds of Formula 3

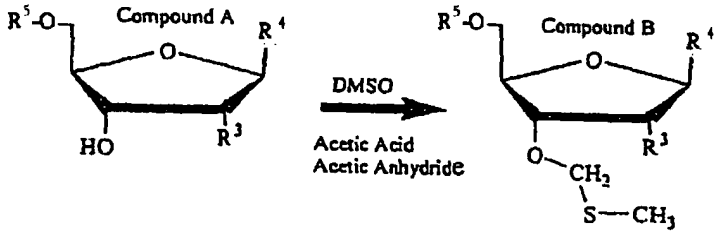


(Formula 3)

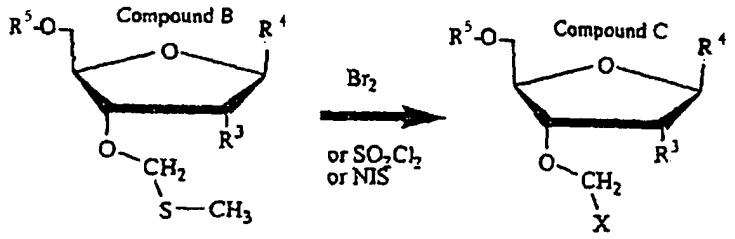
or a salt thereof, wherein R^5 is H, a protecting group, a phosphate group, diphosphate group, or a triphosphate group; R^4 is nucleobase; R^3 is H or OH, or a protected form of OH; and R^9 is H , a heteroatom, a heterogroup, any organic radical having a carbon atom directly attached to the remainder of the molecule, chosen from, saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups or a label-modified hydrocarbyl. R^9 may be used to link the compound of Formula 3 to a solid support.

[0040] A nucleobase containing dithiomethyl-modified compound can be chemically synthesized using the methods described herein. For example:

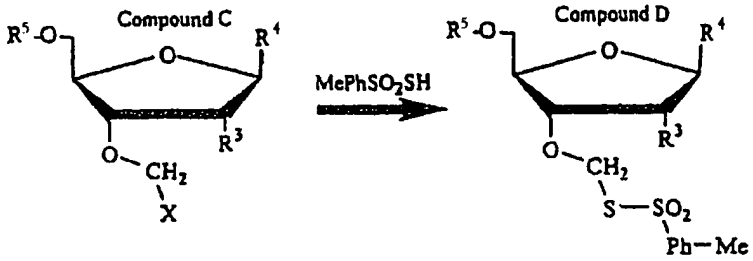
[0041] Compound A (wherein R^1 is a suitable protecting group, R^2 is a nucleobase, and R^3 is either a protected hydroxyl or H) is treated with a mixture of DMSO, acetic acid, and acetic anhydride to form a methylthiomethyl ether (compound B)



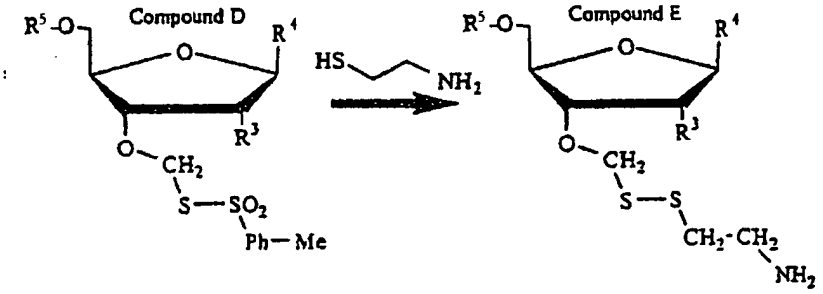
[0042] The methylthiomethyl ether (compound B) is converted to a more reactive halogenated species (compound C) wherein X is Br, Cl, or I.



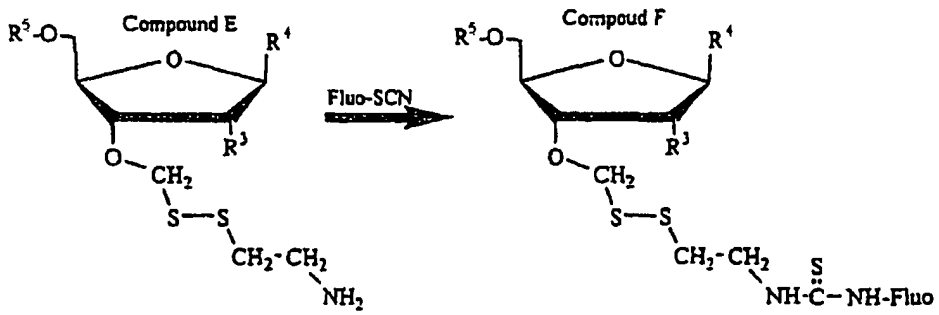
[0043] Compound C is treated with an alkyl- or arylthiosulfonate such as methylphenylthiosulfonate ($MePhSO_2SH$) to prepare compound D.



[0044] Compound D is treated with a hydrocarbylthiol compound such as 2-thioaminoethane to form a dithiomethyl-modified nucleobase (compound E). In some instances, compound E may be the final product.



[0045] When the thiol used to form compound E contains a modifiable substituent, compound E can be further modified or labeled as shown below. Compound E can be treated under known conditions with an isothiocyanate form of a suitable fluorophore (such as fluoresceinisothiocyanate) to form compound F. Compound F can be further modified. For example, R^1 can be replaced with a mono, di, or triphosphate group. Forming a triphosphate can facilitate using the nucleobase containing compounds in enzymatic and template-dependent DNA or RNA synthesis reactions.



[0046] Dithiomethyl-modified nucleotide triphosphates protected at the 3' position (as described above) are useful for any sequencing method. 3'-dithiomethyl-modified nucleotide triphosphates can terminate extension of the primer sequence when used in a DNA polymerase-mediated sequencing method.. Unlike most conventional dideoxy methods where incorporation of the dideoxynucleotide is permanent, however, termination using a dithiomethyl-modified nucleotide is reversible. Thus, one of the benefits associated with using a dithiomethyl-modified nucleotides for sequencing is that the sequencing reaction can be stopped and started by utilizing the labile nature of the protecting group. That is, the dithiomethyl-moiety can be removed by reducing the disulfide bond of the protecting group. Reducing the disulfide creates an unstable intermediate that spontaneously decomposes to produce a free 3' hydroxyl, which can be used for attaching another nucleotide. The disulfide of the dithiomethyl-moiety can be reduced using any reducing agent. Suitable reducing agents include dithiothreitol (DTT), mercaptoethanol, dithionite, reduced glutathione, reduced glutaredoxin, reduced thioredoxin, and any other peptide or organic based reducing agent, or other reagents known to those of ordinary skill in the art. Reduction can be achieved under neutral conditions. It is to be understood that the reduction step leading to the spontaneous decomposition of the intermediate is applicable to all dithiomethyl-modified compounds. Accordingly, it may be necessary to adjust the conditions of conventional sequencing reactions using DNA polymerase enzymes that utilize reduced thioredoxin so that free thiols are not present when the dithiomethyl-modified nucleotides are added.

[0047] The compounds of Formulas 1-3 are useful as reagents in almost any method for sequencing a nucleic acid molecule. General methods for sequencing nucleic acids are known and include dideoxy sequencing methods (Sanger et al., *Proc. Natl. Acad. Sci.*, 74:5463-5467 (1977), chemical degradation methods (Maxam & Gilbert, *Proc. Natl. Acad. Sci.*, 74:560-64 (1977), minisequencing methods (Syvänen et al., *Genomics*, 8:684-92 (1990), and sequencing by synthesis (i.e., multiple iterations of the minisequencing method). It is common practice in these methods to block the 3' hydroxyl of some of the nucleotides. Further, the sequencing by synthesis method requires the availability of nucleotides having a reversibly blocked 3' hydroxyl.

[0048] For example, a sequencing method can proceed by contacting a target nucleic acid with a primer. The target nucleic acid can be any nucleic acid molecule. The primer would also be a nucleic acid molecule. Typically, the primer is shorter than the nucleic acid to be sequenced. Methods for preparing nucleic acids for sequencing and for manufacturing and preparing primer sequences to be used in a sequencing reaction are known. It is advantageous to design the primer so that at least a portion of the primer is complementary to a portion of the target nucleic acid. It is also advantageous to design the primer so that the whole primer is complementary to a portion of the target nucleic acid.

[0049] During a sequencing reaction, the primer and target nucleic acid sequences are combined so that the primer anneals or hybridizes to the target nucleic acid in a sequence specific manner. A DNA polymerase enzyme is then used to incorporate additional nucleotides into the primer in a sequence specific or template-dependent manner such that the nucleotide incorporated into the primer is complementary to the target nucleic acid. For example, a 3'-dithiomethyl-modified nucleotide or a mixture of nucleotides is added to the sequencing reaction at a sufficient concentration so that the DNA polymerase incorporates into the primer a single dithiomethyl-modified nucleotide that is complementary to the target sequence. The incorporation of the dithiomethyl-modified nucleotide can be detected by any known method that is appropriate for the type of label used.

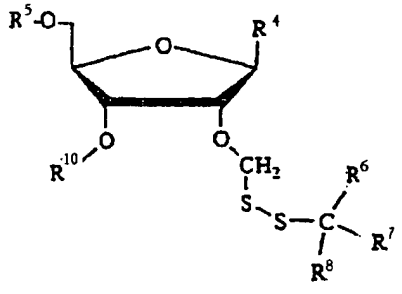
[0050] A second or subsequent round of incorporation for the dithiomethyl-modified nucleotide can occur after incubating the primer target sequence complex with a suitable reducing agent Further, each round of incorporation can be completed without disrupting the hybridization between primer and target sequence. After reduction of the disulfide, the 3'-OH becomes unblocked and ready to accept another round of nucleotide incorporation. The incorporation and reduction steps can be repeated as needed to complete the sequencing of the target sequence. In this way, it may be advantageous to differentially label the individual nucleotides so that incorporation of different nucleotides can be detected. Such a method can be used in single sequencing reactions, automated sequencing reactions, and array based sequencing

reactions.

[0051] Dithiomethyl-modified deoxyribonucleotides and ribonucleotides can also be used for synthesizing oligonucleotides. Chemical synthesis of oligoribonucleotides has an added complexity compared to oligodeoxyribonucleotides due to the presence of the 2'-OH in ribonucleosides. The 2'-OH must be protected during synthesis- Further, the blocking group must be removable during final deblocking. Conditions used for deblocking conventional protecting groups can promote cleavage and/or migration of internucleotide linkages, i.e., the 5'-3' linkage of the oligonucleotide may migrate to form a 5'-2' linkage. This cleavage is both acid and base catalyzed, while migration is acid catalyzed. As such, blocking the 2'-OH with a dithiomethyl moiety is advantageous because the bond is: 1) stable under conventional/standard acidic and basic conditions while other blocked regions of the oligoribonucleotide are deprotected, and 2) the dithiomethyl moiety can be removed under neutral conditions using a simple reducing agent.

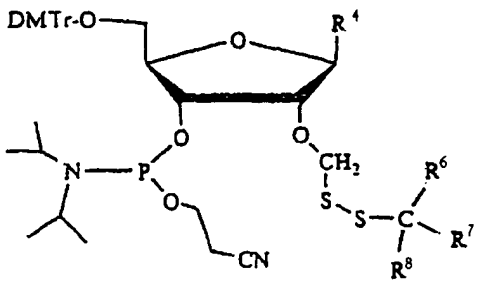
[0052] A method for synthesizing an oligoribonucleotide using ribonucleosides modified at the 2'-OH position with a dithiomethyl moiety can proceed as follows. A first nucleoside is linked to a solid support using known methods. See, e.g., Pon, RT, "Chapter 19 Solid-phase Supports for Oligonucleotide Synthesis," *Methods in Molecular Biology Vol. 20 Protocols for Oligonucleotides and Analogs*, 465-497, Ed. S. Agrawal, Humana Press Inc., Towata, NJ (1993). The 2'-OH modifying moiety can be a dithiomethyl moiety or any other known protecting group (e.g., ester). Alternatively, the first ribonucleoside monomer is linked to a solid support using known methods but also having a linker as shown in Formula 6 (described below). It is to be understood that the 5'-OH and the 3'-OH are also protected as needed using known methods or the methods described herein. After the initial nucleoside is tethered to the solid support, additional dithiomethyl-modified ribonucleoside monomers are added to the growing oligoribonucleotide using any of the existing strategies for internucleotide bond formation. The completed oligoribonucleotide is then deblocked at all positions except the 2'-O- position by using ammonia. The partially deblocked oligoribonucleotide is then contacted with a reducing agent under neutral conditions to achieve the final deprotection. Neutral conditions are conditions that do not promote migration of internucleotide linkages. Conditions having a pH value ranging from about 5 to about 9 and any particular value therebetween, e.g., 7, are considered neutral.

[0053] A suitable ribonucleoside for use in a chemical oligonucleotide synthesis reaction utilizing a dithiomethyl-modified ribonucleoside monomer is shown at Formula 4



(Formula 4)

wherein R^1 is a H or a protecting group, R^2 is a nucleobase, R^6, $R^7$and R^8 are together or separately H or any organic radical having a carbon atom directly attached to the remainder of the molecule, chosen from, saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups. R^{10} may be H, H-phosphonate or phosphoramidite. An example of Formula 4 is shown at Formula 5



(Formula 5)

wherein R^4 is a nucleobase, R^6, R^7 and R^8 are together or separately H or any organic radical having a carbon atom directly attached to the remainder of the molecule, chosen from, saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups.

[0054] Alternatively, other known blocking groups may be used to block the nucleoside at the 5'-OH and 3'-OH positions according the needs of the skilled artisan.

[0055] Suitable ribonucleosides (as described above) can be prepared using known methods and/or the methods described herein for adding a dithiomethyl moiety to a nucleoside.

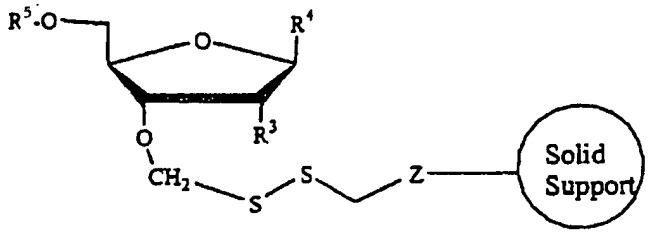
[0056] Chemical synthesis methods using the oligodeoxyribonucleotides and oligoribonucleotides described herein can include methods for inverting the orientation of the oligonucleotides on the solid support. International Application WO 98/51698 entitled "Synthesis of Oligonucleotides" discloses methods for preparing immobilized oligonucleotides and for their subsequent inversion to produce oligonucleotides having a free 3'-OH. These methods together with the compounds and methods described herein can be used together to produce oligonucleotide arrays. The arrays are useful for binding and sequencing reactions, especially automated sequencing reactions.

[0057] Chemical synthesis methods using the oligodeoxyribonucleotides and oligoribonucleotides described herein can include methods for preparing oligonucleotides having different hydrophobic characteristics. Oligonucleotides can be designed to be more or less hydrophobic by using selectively cleavable protecting groups. To alter the hydrophobic character of the nucleotide, a subset of protecting groups is removed after synthesizing the oligonucleotide. By altering the ratio of protecting groups attached the finished oligonucleotide to the number of protecting groups removed, the hydrophobicity of the finished oligonucleotide can be controlled. These types of oligonucleotides can be useful as pro-oligonucleotides for antisense drug treatment methods for a variety of disease states. The article Tasquellas et al., "The Pro-Oligonucleotide Approach: Solid Phase Synthesis And Preliminary Evaluation Of Model Pro-Dodecathymidylates," Nucleic Acids Res. 26:9, 2069-74 (1998) provides an example of such pro-oligonucleotides.

[0058] Oligonucleotides having altered hydrophobicities can be synthesized by following the methods described herein. For example, a first protected nucleoside is covalently attached to a solid support. Additional protected nucleosides are added according to methods described herein to assemble an oligonucleotide. The additional nucleosides may each have different protecting groups. A subset of the protecting groups can be removed. After reducing the dithiobond, the oligonucleotide is removed from the solid phase, washed out and collected. This method allows for isolation of an almost completely protected oligonucleotide. The 3'-terminus of the oligonucleotide can be modified with a reactive or detectable moiety. The oligonucleotide fragments activated at the 3'-position can be used for constructing larger oligonucleotides or synthetic genes. They may also be used in a method for combinatorial synthesis of gene variants lacking any unwanted stop codons.

[0059] Dithiomethyl-modified compounds may also be used to link molecules to a solid support. In particular, it may be advantageous to use dithiomethyl-modified compounds for chemical synthesis of organic molecules such as oligonucleotides, peptides, and carbohydrates. Several methods for coupling organic molecules to solid supports are known-See, e.g., Pon, RT, "Chapter 19 Solid-phase Supports for Oligonucleotide Synthesis," *Methods in Molecular Biology Vol. 20 Protocols for Oligonucleotides and Analogs,* 465-497, Ed. S. Agrawal, Humana Press Inc., Towata, NJ (1993). Only a few methods, however, provide linkages that are inert under acidic and basic conditions, and yield a free hydroxyl group after cleaving the linkage. For example, photochemically labile o-nitrobenzyl ether linkages, siloxyl linkages, and disiloxyl type linkages that are cleavable using fluoride anions, are both inert under acidic and basic conditions and yield a free hydroxyl group after cleaving the linkage. Use of the above linkages is sometimes impractical or associated with unwanted side reactions. The dithiomethyl-modified linkages described herein are cleavable under neutral conditions.

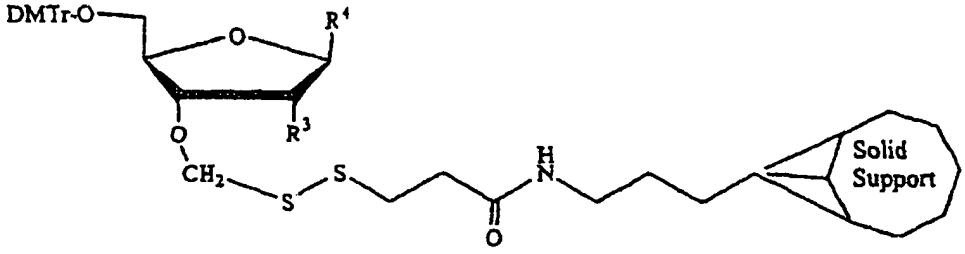
[0060] Accordingly, an oligonucleotide synthesis support can include the molecule shown in Formula 6



(Formula 6)

wherein R^5 is H, phosphate, diphosphate, triphosphate, or a 5'-protecting group, R^4 is a nucleobase, R^3 is H, OH, or a protected form of OH, and Z is a group effective for covalent attachment to a solid support. Examples of Z include amido, ether and any other linking function groups known to those of ordinary skill in the art Such a linker capable of being coupled to a solid support can be effective for securing an oligonucleotide during oligonucleotide synthesis.

[0061] An example of a linker described in Formula 6 is shown in Formula 7



Formula 7

wherein R^4 is a nucleobase, R^3 is H, OH, or a protected form of OH.

[0062] Chemical synthesis of an oligonucleotide can be done by attaching a first nucleoside monomer to a solid support. Any known solid support can be used including non-porous and porous solid supports and organic and inorganic solid supports. Useful solid supports include polystyrenes, cross-linked polystyrenes, polypropylene, polyethylene, teflon, polysaccharides, cross-linked polysaccharides, silica, and various glasses. In some instances, certain solid supports are not fully compatible with aspects of oligonucleotide synthesis chemistry. For example, strong alkaline conditions at elevated temperatures used for deprotection of synthetic oligonucleotides or fluoride anions such as those provided by tetrabutylammonium fluoride cannot be applied to silica or glass supports. Conventional linkers and methods for attaching monomers or oligonucleotides to a solid support are known. See Beaucage & Iyer, *Tetrahedron*, 48(12):2223-2311 (1992).

[0063] The invention will be further described in the following examples, which do not limit the invention as set forth in the claims.

Example 1. Synthesizing 5'-O-FMOC-thymidine.

[0064] Thymidine (10 mmol) was dried by coevaporation with dry pyridine (2 x 30 ml), re-dissolved in dry pyridine (50 ml) and cooled using an acetone/carbon dioxide bath to a temperature of -20° C. The thymidine solution was magnetically stirred and a dichloromethane solution of FMOC-C1 (12 mmol, 1.2 eq. in 20 ml DCM) was added over a period of 60 minutes.. The reaction mixture was warmed to room temperature and stirred for additional 60 minutes. The reaction mixture was partitioned between saturated sodium hydrogen carbonate (250 ml) and dichloromethane (3 x 100 ml). The organic phase was saved, combined, evaporated and dried by coevaporation with toluene (2 x 50 ml) forming an oily residue. A pure product was crystallized from the oily residue using dichloromethane (30 ml) and benzene (50 ml) as solvent. Yield 76 % - white crystals.

Example 2. Synthesizing 5'-O-FMOC-3'-O-methylthiomethyl-thymidine.

[0065] The produce of Example 1 (5'-O-FMOC-Thymidine (7.0 mmol)) was dissolved in 50 ml of an acetic acid:acetic anhydride:DMSO solution (11:35:54, v/v) at 20° C according to (Zavgorodny et al. (1991) *Tetrahedron Lett.* 32: 7593-7596). The solution was stirred at 20° C for 4 days resulting in a complete conversion of the starting material to

methylthiomethyl ether derivative as monitored by thin layer chromatography (TLC). The solvent was evaporated using a rotary evaporator at 50° C under high vacuum (oil pump). The residue was dissolved in ethanol (30 ml) and poured into vigorously stirred water (500 ml). A solid material precipitated and was filtered off. The precipitate was then dissolved in dichloromethane, coevaporated with toluene (2 x 50 ml), and flash chromatographed using dichloromethane: chloroform (1:1 v:v) as the solvent to give the final product as an oil. Yield 72%.

Example 3. Synthesizing 5'-O-FMOC-3'-O-(4-methylphenylthiosulfanatemethyl)-thymidine.

[0066] The product of Example 2 (5'0-FMOC-3'-O-methylthiomethyl-thymidine (4.0 mmol)) was dissolved in a solution of dichloromethane (20 ml) and bromine (Br_2) (226 µl) was added at 20° C. After a 10 minute incubation, a potassium salt of p-toluenethiosulfonic acid (10.0 mmol) dissolved in dry DMF (10 ml) and lutidine (1.5 ml) was added. The reaction mixture was stirred for an additional 120 minutes, quenched by addition of saturated $NaHCO_3$ and extracted with dichloromethane (3 x 50 ml). The resulting organic phase was evaporated, coevaporated with toluene, and flash chromatographed using chloroform as the final solvent. The final product was isolated as an oil. Yield 58%.

Example 4. Synthesizing 3'-O-hydrocarbyldithiomethyl)thymidine derivatives.

[0067] The product of Example 3 (1 mmol) is dissolved in pyridine (5.0 ml) and an appropriate thiol, such as reduced cystamine ("R"SH, i.e., hydrocarbylthiol) (1.1 mmol) dissolved in pyridine (2.0 ml), is added.

[0068] The mixture is stirred for 60 min at 20° C, then extracted using conventional bicarbonate extraction methods and purified by flash chromatography. In some instances it may be advantageous to continue the synthetic process by addition of dry triethylamine (4.0 mmol) in order to remove the 5'-O-FMOC protecting group. After 45 minutes the solvent is evaporated and the 5'-OH derivative is isolated by chromatography after the standard work-up using aqueous $NaHCO_2$ and dichloromethane and evaporating the organic extracts.

Example 5. Synthesizing.3 '-O-(2-N-dansylethyldithiomethyJ)-thymidine.

[0069] The general procedure of Example 4 is followed using N-dansylethanethiol as the thiol. N-dansylethanethiol is prepared by reacting cystamine dihydrochloride with dansyl chloride followed by reducing the disulfide with sodium borohydride. N-dansylethanethiol is isolated using rapid silica gel purification.

[0070] After forming the desired 2-N-dansylethyldithiomethyl linkage, the 5' FMOC group is removed using known methods.

Example 6. Synthesizing 3'-O-(2-N-dansylethyldithiomethyl)-thymidine-5'-triphosphate tetralithium salt.

[0071] The product of Example 5 (3'-O-(2-N-dansylethyldithiomethyl)-thymidine (0.1 mmol)) is dried by coevaporation with dry pyridine (2 x 5 ml) and dissolved in dry acetonitrile (2.0 ml). Phosphorotristriazolide (0.1 M) in dry acetonitrile is prepared from phosphorus oxychloride and triazole as described in Kraszewski & Stawinski, *Tetrahedron Lett.*, 21: 2935-2936 (1980). Phosphorotristriazolide (1.5 ml, 1.5 eq.) is added to the 3'-O-(2-N-dansylethyldithiomethyl)-thymidine at room temperature or 20°C. The mixture is stirred for 5 minutes at which point n-butylammonium pyrophosphate in dry DMF (0.2 M, 1.5 ml, 2.0 eq.) is added. The mixture is stirred overnight at 20° C. Water (2 ml) is then added and hydrolysis of the phosphates occurs (180 min). The nucleotide triphosphate is applied to an anion exchange column Mono Q (TM) (Pharmacia Biotech. Sweden) equilibrated with triethylammonium bicarbonate (TEAB) (0.01 M) and eluted from the Mono Q (TM) column using a linear gradient of TEAB (0.85M):acetonitrile (33 %, v/v). The isolated 3'-O-(2-N-dansylethyldithiomethyl)-thymidine-5'-triphosphate tetralithium salt fraction is evaporated, coevaporated with water and passed through a Dowex 50W x 8 (BDH) in a lithium form to accomplish exchange of the triethylammonium to the lithium ions. At this point the dithiomethyl-modified nucleotide is ready to be used.

[0072] Other aspects, advantages, and modifications are within the scope of the following claims.

Claims

1. A dithiomethyl-modified compound comprising the Formula:

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

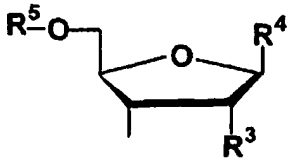
or a salt thereof, wherein

R^1 is chosen from modified or unmodified amino acids, peptides, proteins, carbohydrates, sterols, steroids, ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, base- and/or sugar-modified ribonucleotides, deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides; and

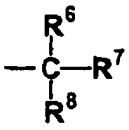
R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups.

2. A compound of claim 1 wherein

R^1 is



R^2 is



R^3 is H, OH, or a protected form of OH;

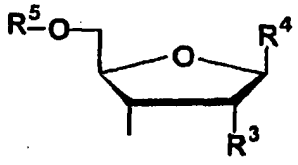
R^4 is a nucleobase;

R^5 is H, a protecting group, phosphate, diphosphate, triphosphate, or residue of a nucleic acid and

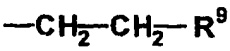
R^6, R^7 and R^8 are together or separately H, a group chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups, or a residue of a solid support.

3. A compound according to claim 1, wherein

R^1 is:



R^2 is



R^3 is H, OH, or a protected form of OH;
R^4 is a nucleobase;
R^5 is H, a protecting group, phosphate, diphosphate, triphosphate, or residue of a nucleic acid and
R^9 is H or a group chosen from saturated and unsaturated hydrocarbons, straight-and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups.

4. The compound of any of claims 1, 2 and 3, wherein said R^1 further comprises at least one hydroxyl group that is not dithiomethyl-modified.

5. The compound of claims 1, wherein said dithiomethyl modification is at a 3' hydroxyl position of said R^1.

6. The compound of claim 5, wherein said dithiomethyl modification is at a 5' hydroxyl position of said R^1.

7. The compound of claim 1 wherein said R^2 comprises a fluorescent labeling group.

8. The compound of claim 7, wherein said fluorescent labeling group is selected from the group consisting of Bodipy™, Dansy™, fluorescein, rhodamin, Texas red™, Cy2™ Cy 4™, and Cy 6™.

9. The compound of claim 1, wherein said R^2 comprises a labeling group.

10. The compound of claim 2, wherein R^6, R^7 and R^8 together or separately further comprise a labelling group.

11. The compound of claim 2, wherein R^6, R^7 and R^8 are together or separately H, methyl, ethyl, isopropyl, t-butyl, phenyl, or benzyl and wherein either R^4, R^5 or R^6 is modified with a labelling group.

12. The compound of claim 3, wherein R^9 is modified with a labeling group.

13. The compound of claim 1, wherein said R^1 is selected from the group consisting of ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, and base- and/or sugar-modified ribonucleotides, and wherein said dithiomethyl modification is at a 2' hydroxyl position of said R^1.

14. The compound of claim 1 wherein said R^2 further comprises an electron donating or withdrawing function.

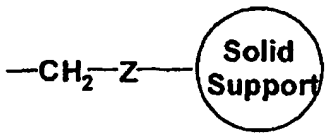
15. The compound of claim 2, wherein R^6, R^7 and R^8 comprise together or separately an electron donating or withdrawing function.

16. The compound of claim 14 and 15, wherein said electron donating or withdrawing function contains a heteroatom selected from the group consisting of oxygen, nitrogen, sulphur, and silicon.

17. The compound of claim 3, wherein R^9 comprises nitrogen.

18. The compound of claim 3, wherein R^9 is covalently linked to a solid support.

19. The compound according to any of claims 3 and 18: wherein R^9 is

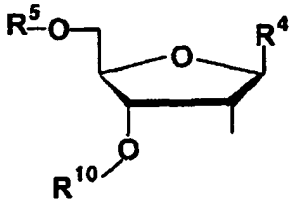


wherein Z is a group effective for covalent attachment to a solid support, said solid support being effective for covalently bonding an oligonucleotide during oligonucleotide synthesis.

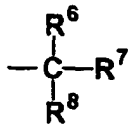
20. The compound of claim 19, wherein said Z is selected from the group consisting of amino, amide, ester, and ether.

21. The compound of claim 1 wherein

R1 is



wherein R^2 is



wherein
R^4 is a nucleobase;
R^5 is H, a protecting group, a phosphate, diphosphate, or a triphosphate, or a residue of a nucleic acid;
R^6, R^7 and R^8 are together or separately H or a group chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups ; and
R^{10} is H, l-l-phosphonate or phosphoramidite.

22. A method for modifying a nucleoside comprising the steps of

a) contacting a nucleoside having at least one hallogenomethyl-modified hydroxyl group with a thiosulfonate compound thereby forming a thiosulfonated nucleoside; and
b) contacting said thiosulfonated nucleoside with a thiol compound thereby forming a dithiomethyl-modified nucleoside with the formula

$$R^1O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wherein

R^1 is selected from the group consisting of ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, base- and/or sugar-modified ribonucleotides, deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides and
R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups.

23. The method of claim 22, wherein said thiosulfonate compound is selected from the group consisting of alkylthiosulfonate and arylthiosulfonate.

24. The method of claim 22 further comprising the step of labelling said dithiomethyl-modified nucleoside.

25. A method for sequencing a nucleic acid comprising the steps of:

a) contacting a target nucleic acid with a primer under conditions wherein said primer anneals to said target nucleic acid in a sequence specific manner and wherein at least a portion of said primer is complementary to a portion of said target nucleic acid;
b) incorporating a dithiomethyl-modified nucleotide with the formula

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wherein

R^1 is selected from the group consisting of ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, base- and/or sugar-modified ribonucleotides, deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides and
R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups, into said primer; and

c) detecting incorporation of said dithiomethyl-modified nucleotide, wherein said dithiomethyl-modified nucleotide is complementary to said target nucleic acid at said dithiomethyl-modified nucleotide's site of incorporation thereby identifying the sequence of one nucleobase of said target nucleic acid.

26. The method of claim 25, wherein said incorporating step is catalysed by a DNA polymerase.

27. The method of claim 25, wherein said sequencing method is selected from the group consisting of minisequencing and sequencing by synthesis.

28. The method of claim 25, comprising the steps of incorporating a first 3'-dithiomethyl-modified nucleotide into said primer in step b);

c) detecting said incorporation of said first 3'-dithiomethyl-modified nucleotide thereby identifying the sequence of a nucleobase of said target nucleic acid;
d) removing said dithiomethyl group from said first incorporated dithiomethyl-modified nucleotide to form a first elongated primer having a free hydroxyl group;
e) incorporating a second 3'- dithiomethyl-modified nucleotide into said first elongated primer; and
f) detecting said second dithiomethyl-modified nucleotide thereby identifying the sequence of another nucleobase of said target nucleic acid, wherein said first 3'-dithiomethyl-modified nucleotide and said second 3'-dithiomethyl-modified nucleotide are complementary to said target nucleic acid at each said nucleotide's site of incorporation.

29. The method of claim 28, wherein said detecting steps are performed after removing said dithiomethyl group.

30. The method of claim 25 and 28, wherein said method is effective for use with a sequencing array.

31. The method according to claim 25 comprising the steps of:

a) providing a primer array comprising a plurality of sequencing primers;
b) contacting a target nucleic acid with said primer array under conditions wherein said sequencing primers anneal to said target nucleic acid in a sequence specific manner thereby forming target-primer complexes between complementary portions of said sequencing primers and said target nucleic acid;
c) incorporating a first 3'- dithiomethyl-modified nucleotide into at least one sequencing primer portion of said

target-primer complexes, said first 3'-dithiomethyl-modified nucleotide being complementary to said target nucleic acid; and
d) detecting said incorporation of said first 3'-dithiomethyl-modified nucleotide, wherein said first 3'-dithiomethyl-modifed nucleotide is complementary to said target sequence at said first 3'- dithiomethyl-modified nucleotide's site of incorporation.

32. The method of claim 31 further comprising the steps of:

e) removing said dithiomethyl group from said first incorporated 3'-dithiomethyl-modified nucleotide to form a first elongated target-primer complex having a free 3' hydroxyl group;
f) incorporating a second dithiomethyl-modified nucleotide into said first elongated target-primer complex; and
g) detecting said second 3'-dithiomethyl-modified nucleotide, wherein said second 3'-dithiomethyl-modified nucleotide is complementary to said target sequence at said second 3'-dithiomethyl-modified nucleotide's site of incorporation.

33. The method of claim 25 and 31, wherein said detecting steps are performed before removing said dithiomethyl group.

34. The method of claim 28, wherein said detecting steps are performed after removing said dithiomethyl group.

35. The method of claim 28, wherein steps a), b), c), d), e), and f) are performed under conditions that do not disrupt the annealing of said primer to said target nucleic acid

36. The method of claim 31, wherein said method is effective for producing a plurality of nucleotide sequences, said nucleotide sequences corresponding to overlapping nucleotide sequences of said target nucleic acid.

37. The method of claim 31, wherein said step e) is performed under conditions that do not disrupt said target-primer complexes.

38. A method for synthesizing an oligonucleotide comprising the steps of:

a) providing a 5' protected first nucleoside covalently bonded to a solid support through a linker;
b) deprotecting said first nucleoside at its 5' position;
c) covalently bonding another 5' protected nucleoside to said first nucleotide at the 5' position of said first nucleoside;
d) deprotecting said another nucleoside at its 5' position; and
e) repeating steps c) and d) for adding additional protected nucleosides, said linker securing said first nucleotide to said solid support via a dithiomethyl bond forming a compound with the formula

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wherein

R^1 is selected from the group consisting of deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides and R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups.

39. The method for according to claim 38, wherein the linker is a dithiomethyl containing linker and after step d) the following steps are performed:

e) optionally repeating steps c) and d) for adding additional protected nucleosides thereby producing an oligonucleotide;
f) optionally selectively cleaving a protecting group from said oligonucleotide thereby forming a partially deprotected oligonucleotide;
g) selectively cleaving said dithiomethyl containing linker; and
h) isolating said partially deprotected oligonucleotide.

40. The method of claim 39, wherein said method is effective for inverting said oligonucleotide thereby forming an oligonucleotide having a free 3' hydroxyl and being covalently linked to a solid support.

41. The method of claim 38 and 40, wherein said method is optimised for use in an array.

42. The method of claim 38, further comprising the step of cleaving said oligonucleotide from said solid support.

43. The method of claim 39, further comprising the step of modifying the 3' terminus of said oligonucleotide with a reactive or detectable moiety.

44. The method of claim 39, wherein at least one of said 5' protected nucleosides comprises a dithiomethyl moiety.

45. A method for synthesizing an oligoribonucleotide comprising the steps of:

a) providing a first protected ribonucleoside covalently bonded to a solid support;
b) covalently linking at least one 2'-dithiomethyl-modified ribonucleoside with the formula

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

wherein

R^1 is selected from the group consisting of ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, base- and/or sugar-modified ribonucleotides, and R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying groups ; to said first ribonucleoside to form an oligoribonucleotide;

c) partially deprotecting said oligoribonucleotide under acidic or basic conditions; and
d) contacting said oligoribonucleotide with a reducing agent under neutral conditions thereby deprotecting said 2'-dithiomethyl-modified ribonucleoside in said oligoribonucleotide.

46. The method of claim 45, wherein said 2'-dithiomethyl-modified ribonucleoside is completely deprotected in said oligoribonucleotide.

47. The method of claim 45, wherein the pH of said neutral conditions ranges from about 5 to about 9.

48. The method of claim 47, wherein said pH is about 7.

49. The method of claim 45, wherein said method is effective for inverting said oligoribonucleotide thereby forming a solid phase bound oligoribonucleotide having a free 3' hydroxyl.

50. The method of claim 45, wherein said first protected ribonucleoside is secured to said solid support via a dithiomethyl bond.

51. Use of a dithiomethyl-modified compound comprising the Formula:

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

or a salt thereof, wherein

R^1 is chosen from modified or unmodified amino acids, peptides, proteins, carbohydrates, sterols, steroids ribonucleosides, ribonucleotides, base- and/or sugar-modified ribonucleosides, base- and/or sugar-modified ribonucleotides, deoxyribonucleosides, deoxyribonucleotides, base- and/or sugar-modified deoxyribonucleosides, and base- and/or sugar-modified deoxyribonucleotides; and
R^2 is chosen from saturated and unsaturated hydrocarbons, straight- and branched-chain aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, heterocyclic hydrocarbons, heteroaromatic hydrocarbons, and substituted hydrocarbons such as hydrocarbons containing heteroatoms and/or other functional modifying

groups for sequencing nucleic acids.

Patentansprüche

1. Dithiomethylmodifizierte Verbindung umfassend die Formel:

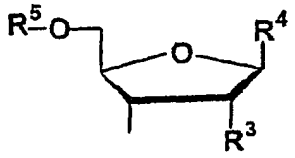
$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

oder ein Salz davon, wobei

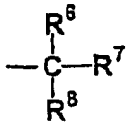
R^1 ausgewählt ist aus modifizierten oder unmodifizierten Aminosäuren, Peptiden, Proteinen, Kohlenhydraten, Sterinen, Steroiden, Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden, basen- und/oder zuckermodifizierten Ribonucleotiden, Desoxyribonucleosiden, Desoxyribonucleotiden, basen- und/oder zuckermodifizierten Desoxyribonucleosiden und basen- und/oder zuckermodifizierten Desoxyribonucleotiden; und
R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten.

2. Verbindung nach Anspruch 1, wobei

R^1



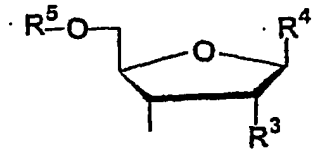
ist,
R^2



ist,
R^3 H, OH oder eine geschützte Form von OH ist;
R^4 eine Nucleobase ist;
R^5 H, eine Schutzgruppe, ein Phosphat, ein Diphosphat, ein Triphosphat oder ein Rest einer Nucleinsäure ist und R^6, R^7 und R^8 gleichzeitig oder unabhängig voneinander H, eine Gruppe, ausgewählt aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten, oder ein Rest eines festen Trägers sind.

3. Verbindung nach Anspruch 1, wobei

R^1



ist,

R^2

$-CH_2-CH_2-R^9$

ist,

R^3 H, OH oder eine geschützte Form von OH ist;

R^4 eine Nucleobase ist;

R^5 H, eine Schutzgruppe, ein Phosphat, ein Diphosphat, ein Triphosphat oder ein Rest einer Nucleinsäure ist, und

R^9 H oder eine Gruppe ist, die ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten.

4. Verbindung nach irgendeinem der Ansprüche 1, 2 und 3, wobei R^1 ferner wenigstens eine Hydroxygruppe umfasst, die nicht dithiomethylmodifiziert ist.

5. Verbindung nach Anspruch 1, wobei sich die Dithiomethylmodifikation an einer 3'-Hydroxyposition von R^1 befindet.

6. Verbindung nach Anspruch 5, wobei sich die Dithiomethylmodifikation an einer 5'-Hydroxyposition von R^1 befindet.

7. Verbindung nach Anspruch 1, wobei R^2 eine fluoreszierende Markergruppe umfasst.

8. Verbindung nach Anspruch 7, wobei die fluoreszierende Markergruppe ausgewählt ist aus der Gruppe bestehend aus Bodipy™, Dansyl™, Fluorescein, Rhodamin, Texas Red™, Cy 2™ Cy 4™ und Cy 6™.

9. Verbindung nach Anspruch 1, wobei R^2 eine Markergruppe umfasst.

10. Verbindung nach Anspruch 2, wobei R^6, R^7 und R^8 gleichzeitig oder unabhängig voneinander außerdem eine Markergruppe umfassen.

11. Verbindung nach Anspruch 2, wobei R^6, R^7 und R^8 gleichzeitig oder unabhängig voneinander H, Methyl, Ethyl, Isopropyl, tert.-Butyl, Phenyl oder Benzyl sind, und wobei entweder R^4, R^5 oder R^6 mit einer Markergruppe modifiziert ist.

12. Verbindung nach Anspruch 3, wobei R^9 mit einer Markergruppe modifiziert ist.

13. Verbindung nach Anspruch 1, wobei R^1 ausgewählt ist aus der Gruppe bestehend aus Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden und basen- und/oder zuckermodifizierten Ribonucleotiden, und wobei sich die Dithiomethylmodifikation an einer 2'-Hydroxyposition von R^1 befindet.

14. Verbindung nach Anspruch 1, wobei R^2 ferner eine elektronenschiebende oder -ziehende Funktion umfasst.

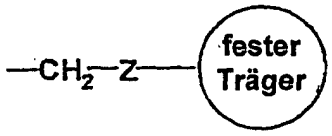
15. Verbindung nach Anspruch 2, wobei R^6, R^7 und R^8 gleichzeitig oder unabhängig voneinander eine elektronenschiebende oder -ziehende Funktion umfassen.

16. Verbindung nach Anspruch 14 und 15, wobei die elektronenschiebende oder -ziehende Funktion ein Heteroatom enthält, ausgewählt aus der Gruppe bestehend aus Sauerstoff, Stickstoff, Schwefel und Silicium.

17. Verbindung nach Anspruch 3, wobei R^9 Stickstoff umfasst.

18. Verbindung nach Anspruch 3, wobei R^9 kovalent an einen festen Träger gebunden ist.

19. Verbindung nach irgendeinem der Ansprüche 3 und 18, wobei R^9

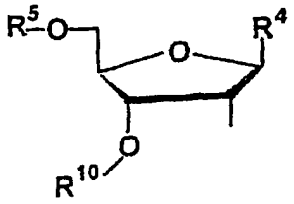


ist, wobei Z eine zur kovalenten Bindung an einen festen Träger geeignete Gruppe ist, wobei der feste Träger zur kovalenten Bindung eines Oligonucleotids während einer Oligonucleotidsynthese in der Lage ist.

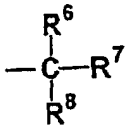
20. Verbindung nach Anspruch 19, wobei Z ausgewählt ist aus der Gruppe bestehend aus Amino, Amid, Ester und Ether.

21. Verbindung nach Anspruch 1, wobei

R^1



ist,
wobei R^2



ist,
wobei R^4 eine Nucleobase ist;
R5 H, eine Schutzgruppe, ein Phosphat, ein Diphosphat oder ein Triphosphat oder ein Rest einer Nucleinsäure ist;
R^6, R^7 und R^8 gleichzeitig oder unabhängig voneinander H oder eine Gruppe sind, die ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten; und
R^{10} H, H-Phosphonat oder Phosphoramidit ist.

22. Verfahren zum Modifizieren eines Nucleosids, umfassend die Schritte:

a) In-Kontakt-Bringen eines Nucleosids, das wenigstens eine halogenmethylmodifizierte Hydroxygruppe aufweist, mit einer Thiosulfonatverbindung unter Bildung eines thiosulfonierten Nucleosids; und
b) In-Kontakt-Bringen dieses thiosulfonierten Nucleosids mit einer Thiolverbindung unter Bildung eines dithio-

methylmodifizierten Nucleosids der Formel

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wobei

R^1 ausgewählt ist aus der Gruppe bestehend aus Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden, basen- und/oder zuckermodifizierten Ribonucleotiden, Desoxyribonucleosiden, Desoxyribonucleotiden, basen- und/oder zuckermodifizierten Desoxyribonucleosiden und basen- und/oder zukkermodifizierten Desoxyribonucleotiden; und
R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten.

23. Verfahren nach Anspruch 22, wobei die Thiosulfonatverbindung ausgewählt ist aus der Gruppe bestehend aus Alkylthiosulfonat und Arylthiosulfonat.

24. Verfahren nach Anspruch 22, ferner umfassend den Schritt des Markierens dieses dithiomethylmodifizierten Nucleosids.

25. Verfahren zum Sequenzieren einer Nucleinsäure, umfassend die Schritte:

a) In-Kontakt-Bringen einer Zielnucleinsäure mit einem Primer unter Bedingungen, bei denen der Primer sequenzspezifisch an die Zielnucleinsäure hybridisiert, und wobei wenigstens ein Teil des Primers zu einem Teil der Zielnucleinsäure komplementär ist;
b) Einbau eines dithiomethylmodifizierten Nucleotids der Formel

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wobei

R^1 ausgewählt ist aus der Gruppe bestehend aus Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden, basen- und/oder zuckermodifizierten Ribonucleotiden, Desoxyribonucleosiden, Desoxyribonucleotiden, basen- und/oder zuckermodifizierten Desoxyribonucleosiden und basen- und/oder zukkermodifizierten Desoxyribonucleotiden; und
R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten,

in den Primer; und
c) Nachweis des Einbaus dieses dithiomethylmodifizierten Nucleotids, wobei das dithiomethylmodifizierte Nucleotid an der Einbaustelle des dithiomethylmodifizierten Nucleotids zu der Zielnucleinsäure komplementär ist, womit die Sequenz einer Nucleobase der Zielnucleinsäure bestimmt wird.

26. Verfahren nach Anspruch 25, wobei der Einbauschritt durch eine DNA-Polymerase katalysiert wird.

27. Verfahren nach Anspruch 25, wobei das Sequenzierverfahren ausgewählt ist aus der Gruppe bestehend aus Minisequenzierung und Sequenzierung durch Synthese.

28. Verfahren nach Anspruch 25, umfassend die Schritte des Einbaus eines ersten 3'-dithiomethylmodifizierten Nucleotids in den Primer in Schritt b);

c) Nachweis des Einbaus dieses ersten 3'-dithiomethylmodifizierten Nucleotids, womit die Sequenz einer Nucleobase der Zielnucleinsäure bestimmt wird;

d) Entfernen der Dithiomethylgruppe von dem ersten eingebauten dithiomethylmodifizierten Nucleotid unter Bildung eines ersten verlängerten Primers mit einer freien Hydroxygruppe;
e) Einbau eines zweiten 3'-dithiomethylmodifizierten Nucleotids in den ersten verlängerten Primer; und
f) Nachweis des zweiten dithiomethylmodifizierten Nucleotids, womit die Sequenz einer weiteren Nucleobase der Zielnucleinsäure bestimmt wird, wobei das erste 3'-dithiomethylmodifizierte Nucleotid und das zweite 3'-dithiomethylmodifizierte Nucleotid an jeder der Einbaustellen dieser Nucleotide zu der Zielnucleinsäure komplementär sind.

29. Verfahren nach Anspruch 28, wobei die Nachweisschritte nach Entfernen der Dithiomethylgruppe durchgeführt werden.

30. Verfahren nach Anspruch 25 und 28, wobei das Verfahren zur Verwendung bei einem Sequenzierungsarray geeignet ist.

31. Verfahren nach Anspruch 25, umfassend die Schritte:

a) Bereitstellen eines Primerarrays, der eine Vielzahl Sequenzierprimer umfasst;
b) In-Kontakt-Bringen einer Zielnucleinsäure mit dem Primerarray unter Bedingungen, bei denen die Sequenzierprimer sequenzspezifisch an die Zielnucleinsäure hybridisieren und **dadurch** Ziel-Primer-Komplexe zwischen komplementären Teilen der Sequenzierprimer und der Zielnucleinsäure bilden;
c) Einbau eines ersten 3'-dithiomethylmodifizierten Nucleotids in wenigstens einen Sequenzierprimerteil der Ziel-Primer-Komplexe, wobei das erste 3'-dithiomethylmodifizierte Nucleotid zu der Zielnucleinsäure komplementär ist; und
d) Nachweis des Einbaus des ersten 3'-dithiomethylmodifizierten Nucleotids, wobei das erste 3'-dithiomethylmodifizierte Nucleotid an der Einbaustelle des ersten 3'-dithiomethylmodifizierten Nucleotids zu der Zielsequenz komplementär ist.

32. Verfahren nach Anspruch 31, ferner umfassend die Schritte:

e) Entfernen der Dithiomethylgruppe von dem ersten eingebauten 3'-dithiomethylmodifizierten Nucleotid unter Bildung eines ersten verlängerten Ziel-Primer-Komplexes mit einer freien 3'-Hydroxygruppe;
f) Einbau eines zweiten dithiomethylmodifizierten Nucleotids in den ersten verlängerten Ziel-Primer-Komplex; und
g) Nachweis des zweiten 3'-dithiomethylmodifizierten Nucleotids, wobei das zweite 3'-dithiomethylmodifizierte Nucleotid an der Einbaustelle des zweiten 3'-dithiomethylmodifizierten Nucleotids zu der Zielsequenz komplementär ist.

33. Verfahren nach Anspruch 25 und 31, wobei die Nachweisschritte vor Entfernen der Dithiomethylgruppe durchgeführt werden.

34. Verfahren nach Anspruch 28, wobei die Nachweisschritte nach Entfernen der Dithiomethylgruppe durchgeführt werden.

35. Verfahren nach Anspruch 28, wobei die Schritte a), b), c), d), e) und f) unter Bedingungen durchgeführt werden, die die Hybridisierung des Primers an die Zielnucleinsäure nicht stören.

36. Verfahren nach Anspruch 31, wobei sich das Verfahren zur Herstellung einer Vielzahl von Nucleotidsequenzen eignet, wobei die Nucleotidsequenzen überlappenden Nucleotidsequenzen der Zielnucleinsäure entsprechen.

37. Verfahren nach Anspruch 31, wobei Schritt e) unter Bedingungen durchgeführt wird, die die Ziel-Primer-Komplexe nicht zerstören.

38. Verfahren zur Synthese eines Oligonucleotids, umfassend die Schritte:

a) Bereitstellen eines 5'-geschützten ersten Nucleosids, das über einen Linker kovalent an einen festen Träger gebunden ist;
b) Entschützen des ersten Nucleosids an dessen 5'-Position.
c) kovalentes Binden eines weiteren 5'-geschützten Nucleosids an das erste Nucleotid an der 5'-Position des

ersten Nucleosids;
d) Entschützen dieses weiteren Nucleosids an dessen 5'-Position; und
e) Wiederholen der Schritte c) und d) zum Anhängen weiterer geschützter Nucleoside, wobei der Linker das erste Nucleotid über eine Dithiomethylbindung an dem festen Träger befestigt, wobei eine Verbindung der Formel

$$R1\text{-}O\text{-}H_2\text{-}S\text{-}S\text{-}R^2$$

gebildet wird, in der

R^1 ausgewählt ist aus der Gruppe bestehend aus Desoxyribonucleosiden, Desoxyribonucleotiden, basen- und/oder zuckermodifizierten Desoxyribonucleosiden und basen- und/oder zuckermodifizierten Desoxyribonucleotiden, und R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten.

39. Verfahren nach Anspruch 38, wobei der Linker ein Linker ist, der Dithiomethyl enthält, und nach Schritt d) die folgenden Schritte durchgeführt werden:

e) gegebenenfalls Wiederholen der Schritte c) und d) zum Anhängen weiterer geschützter Nucleoside, womit ein Oligonucleotid gebildet wird;
f) gegebenenfalls selektives Abspalten einer Schutzgruppe von dem Oligonucleotid, womit ein partiell entschützten Oligonucleotid gebildet wird;
g) selektives Abspalten des Dithiomethyl enthaltenden Linkers; und
h) Isolieren des partiell entschützten Oligonucleotids.

40. Verfahren nach Anspruch 39, wobei das Verfahren zum Invertieren des Oligonucleotids geeignet ist, womit ein Oligonucleotid gebildet wird, das eine freie 3'-Hydroxygruppe besitzt und kovalent an einen festen Träger gebunden ist.

41. Verfahren nach Anspruch 38 und 40, wobei das Verfahren zur Verwendung bei einem Array optimiert ist.

42. Verfahren nach Anspruch 38, ferner umfassend den Schritt des Abspaltens des Oligonucleotids von dem festen Träger.

43. Verfahren nach Anspruch 39, ferner umfassend den Schritt des Modifizierens des 3'-Terminus dieses Oligonucleotids mit einer reaktiven oder nachweisbaren Funktion.

44. Verfahren nach Anspruch 39, wobei wenigstens eines der 5'-geschützten Nucleoside eine Dithiomethylfunktion umfasst.

45. Verfahren zur Synthese eines Oligoribonucleotids, umfassend die Schritte:

a) Bereitstellen eines ersten geschützten Ribonucleosids, das kovalent an einen festen Träger gebunden ist;
b) kovalentes Binden wenigstens eines 2'-dithiomethylmodifizierten Ribonucleosids der Formel

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

wobei

R^1 ausgewählt ist aus der Gruppe bestehend aus Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden, basen- und/oder zuckermodifizierten Ribonucleotiden, und R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwassersfioffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten;

an das erste Ribonucleosid unter Bildung eines Oligoribonucleotids;
c) partielles Entschützen des Oligoribonucleotids unter sauren oder basischen Bedingungen; und
d) In-Kontakt-Bringen des Oligoribonucleotids mit einem Reduktionsmittel unter neutralen Bedingungen und **dadurch** Entschützen des 2'-dithiomethylmodifizierten Ribonucleosids in dem Oligoribonucleotid.

46. Verfahren nach Anspruch 45, wobei das 2'-dithiomethylmodifizierte Ribonucleosid in dem Oligoribonucleotid vollständig entschützt wird.

47. Verfahren nach Anspruch 45, wobei der pH der neutralen Bedingungen im Bereich von etwa 5 bis etwa 9 liegt.

48. Verfahren nach Anspruch 47, wobei der pH etwa 7 ist.

49. Verfahren nach Anspruch 45, wobei das Verfahren zum Invertieren des Oligoribonucleotids geeignet ist, womit ein festphasengebundenes Oligoribonucleotid mit einem freien 3'-Hydroxy gebildet wird.

50. Verfahren nach Anspruch 45, wobei das erste geschützte Ribonucleosid über eine Dithiomethylbindung an dem festen Träger befestigt ist.

51. Verwendung einer dithiomethylmodifizierten Verbindung umfassend die Formel

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

oder eines Salzes davon, wobei

R^1 ausgewählt ist aus modifizierten oder unmodifizierten Aminosäuren, Peptiden, Proteinen, Kohlenhydraten, Sterinen, Steroiden, Ribonucleosiden, Ribonucleotiden, basen- und/oder zuckermodifizierten Ribonucleosiden, basen- und/oder zuckermodifizierten Ribonucleotiden, Desoxyribonucleosiden, Desoxyribonucleotiden, basen- und/oder zuckermodifizierten Desoxyribonucleosiden und basen- und/oder zuckermodifizierten Desoxyribonucleotiden; und
R^2 ausgewählt ist aus gesättigten und ungesättigten Kohlenwasserstoffen, gerad- und verzweigtkettigen aliphatischen Kohlenwasserstoffen, cyclischen Kohlenwasserstoffen, aromatischen Kohlenwasserstoffen, heterocyclischen Kohlenwasserstoffen, heteroaromatischen Kohlenwasserstoffen und substituierten Kohlenwasserstoffen, wie Kohlenwasserstoffen, die Heteroatome und/oder andere funktionelle modifizierende Gruppen enthalten, zum Sequenzieren von Nucleinsäuren.

Revendications

1. Composé modifié par un radical dithiométhyle comprenant à la formule :

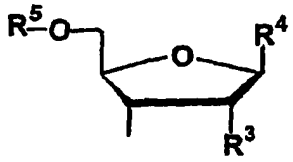
$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

ou un sel de celui-ci, dans laquelle

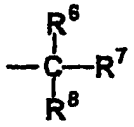
R^1 est choisi parmi des acides aminés modifiés ou non modifiés, des peptides, des protéines, des glucides, des stérols, des stéroïdes, des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, des ribonucléotides ayant une base et/ou un sucre modifiés, des désoxyribonucléosides, des désoxyribonucléotides, des désoxyribonucléosides ayant une base et/ou un sucre modifiés, et des désoxyribonucléotides ayant une base et/ou un sucre modifiés ; et
R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification.

2. Composé selon la revendication 1, dans lequel

R^1 est :



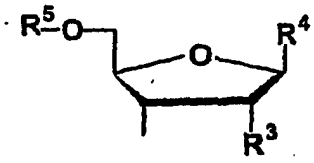
R^2est:



R^3 est H, OH, ou une forme protégée de OH ;
R^4 est une nucléobase ;
R^5 est H, un groupe de protection, un phosphate, un diphosphate, un triphosphate, ou un résidu d'un acide nucléique, et
R^6, R^7 et R^8 sont ensemble ou séparément H, un groupe choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification, ou un résidu d'un support solide.

3. Composé selon la revendication 1, dans lequel

R^1 est :



R^2 est:

$$-CH_2-CH_2-R^9$$

R^3 est H, OH, ou une forme protégée de OH ;
R^4 est une nucléobase ;
R^5 est H, un groupe de protection, un phosphate, un diphosphate, un triphosphate, ou un résidu d'un acide nucléique, et
R^9 est H ou un groupe choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification.

4. Composé selon l'une quelconque des revendications 1, 2 et 3, dans lequel ledit R^1 comprend de plus au moins un groupe hydroxyle qui n'est pas modifié par un radical dithiométhyle.

5. Composé selon la revendication 1, dans lequel ladite modification par un radical dithiométhyle se situe au niveau d'un hydroxyle en position 3' dudit R^1.

6. Composé selon la revendication 5, dans lequel ladite modification par un radical dithiométhyle se situe au niveau d'un hydroxyle en position 5' dudit R^1.

7. Composé selon la revendication 1, dans lequel ledit R^2 comprend un groupe de marquage fluorescent.

8. Composé selon la revendication 7, dans lequel ledit groupe de marquage fluorescent est choisi dans le groupe consistant en Bodipy™, Dansyl™, fluorescéine, rhodamine, Texasred™, Cy 2™, Cy 4™, et Cy 6™.

9. Composé selon la revendication 1, dans lequel ledit R^2 comprend un groupe de marquage.

10. Composé selon la revendication 2, dans lequel R^6, R^7 et R^8 comprennent de plus ensemble ou séparément un groupe de marquage.

11. Composé selon la revendication 2, dans lequel R^6, R^7 et R^8 sont ensemble ou séparément H, méthyle, éthyle, isopropyle, t-butyle, phényle, ou benzyle et dans lequel l'un ou l'autre de R^4, R^5, ou R^6 est modifié par un groupe de marquage.

12. Composé selon la revendication 3, dans lequel R^9 est modifié par un groupe de marquage.

13. Composé selon la revendication 1, dans lequel ledit R^1 est choisi dans le groupe consistant en des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, et des ribonucléotides ayant une base et/ou un sucre modifiés, et dans lequel ladite modification par un radical dithiométhyle se situe au niveau d'un hydroxyle en position 2' dudit R^1.

14. Composé selon la revendication 1, dans lequel ledit R2 comprend de plus une fonction de donneur d'électron ou d'accepteur d'électron.

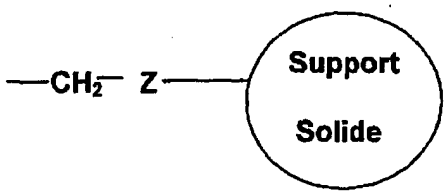
15. Composé selon la revendication 2, dans lequel R^6, R^7 et R^8 comprennent ensemble ou séparément une fonction de donneur d'électron ou d'accepteur d'électron.

16. Composé selon les revendications 14 et 15, dans lequel ladite fonction de donneur d'électron ou d'accepteur d'électron contient un hétéroatome choisi dans le groupe consistant en l'oxygène, l'azote, le soufre, et le silicium.

17. Composé selon la revendication 3, dans lequel R^9 comprend de l'azote.

18. Composé selon la revendication 3, dans lequel R^9 est lié de manière covalente à un support solide.

19. Composé selon l'une quelconque des revendications 3 et 18, dans lequel R^9 est :

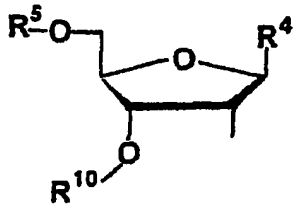


dans lequel Z est un groupe efficace pour une liaison covalente à un support solide, ledit support solide étant efficace pour lier de manière covalente un oligonucléotide au cours d'une synthèse d'oligonucléotide.

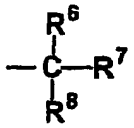
20. Composé selon la revendication 19, dans lequel ledit Z et choisi dans le groupe consistant en amino, amide, ester, et éther.

21. Composé selon la revendication 1, dans lequel

R^1 est :



dans lequel R^2 est :



dans lequel
R^4 est une nucléobase ;
R^5 est H, un groupe de protection, un phosphate, un diphosphate, ou un triphosphate, ou un résidu d'un acide nucléique ;
R^6, R^7 et R^8 sont ensemble ou séparément H ou un groupe choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification ; et
R^{10} est H, H-phosphonate ou phosphoramidite.

22. Procédé de modification d'un nucléoside comprenant les étapes :

a) de mise en contact d'un nucléoside possédant au moins un groupe hydroxyle modifié par un radical halométhyle avec un composé thiosulfonate, formant de ce fait un nucléoside thiosulfonaté ; et
b) de mise en contact dudit nucléoside thiosulfonaté avec un composé thiol, formant de ce fait un nucléoside modifié par un radical dithiométhyle répondant à la formule :

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

dans laquelle

R^1 est choisi dans le groupe consistant en des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, des ribonucléotides ayant une base et/ou un sucre modifiés, des désoxyribonucléosides, des désoxyribonucléotides, des désoxyribonucléosides ayant une base et/ou un sucre modifiés, et des désoxyribonucléotides ayant une base et/ou un sucre modifiés ; et
R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification.

23. Procédé selon la revendication 22, dans lequel ledit composé thiosulfonate est choisi dans le groupe consistant en un alkylthiosulfonate et un arylthiosulfonate.

24. Procédé selon la revendication 22, comprenant de plus l'étape de marquage dudit nucléoside modifié par un radical dithiométhyle.

25. Procédé de séquençage d'un acide nucléique comprenant les étapes :

a) de mise en contact d'un acide nucléique cible avec une amorce dans des conditions dans lesquelles ladite amorce s'hybride audit acide nucléique cible selon une séquence spécifique et dans laquelle au moins une partie de ladite amorce est complémentaire d'une partie dudit acide nucléique cible ;
b) d'incorporation d'un nucléotide modifié par un radical dithiométhyle répondant à la formule :

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

dans laquelle

R^1 est choisi dans le groupe consistant en des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, des ribonucléotides ayant une base et/ou un sucre modifiés, des désoxyribonucléosides, des désoxyribonucléotides, des désoxyribonucléosides ayant une base et/ou un sucre modifiés, et des désoxyribonucléotides ayant une base et/ou un sucre modifiés, et
R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification,

dans ladite amorce ; et
c) de détection de l'incorporation dudit nucléotide modifié par un radical dithiométhyle, dans laquelle ledit nucléotide modifié par un radical dithiométhyle est complémentaire dudit acide nucléique cible au niveau du site d'incorporation dudit nucléotide modifié par un radical dithiométhyle, identifiant de ce fait la séquence d'une nucléobase dudit acide nucléique cible.

26. Procédé selon la revendication 25, dans lequel ladite étape d'incorporation est catalysée par une ADN polymérase.

27. Procédé selon la revendication 25, dans lequel ledit procédé de séquençage est choisi dans le groupe consistant en un mini séquençage et un séquençage par synthèse.

28. Procédé selon la revendication 25, comprenant les étapes d'incorporation d'un premier nucléotide modifié par un radical dithiométhyle en position 3' dans ladite amorce dans l'étape b) ;

c) de détection de ladite incorporation dudit premier nucléotide modifié par un radical dithiométhyle en position 3', identifiant de ce fait la séquence d'une nucléobase dudit acide nucléique cible ;
d) d'élimination dudit groupe dithiométhyle dudit premier nucléotide modifié par un radical dithiométhyle incorporé pour former une première amorce allongée possédant un groupe hydroxyle libre;
e) d'incorporation d'un deuxième nucléotide modifié par un radical dithiométhyle en position 3' dans ladite première amorce allongée ; et
f) de détection dudit deuxième nucléotide modifié par un radical dithiométhyle, identifiant de ce fait la séquence d'une autre nucléobase dudit acide nucléique cible, dans laquelle ledit premier nucléotide modifié par un radical dithiométhyle en position 3' et ledit deuxième nucléotide modifié par un radical dithiométhyle en position 3' sont complémentaires dudit acide nucléique cible au niveau de chacun desdits sites d'incorporation des nucléotides.

29. Procédé selon la revendication 28, dans lequel lesdites étapes de détection sont réalisées après l'élimination dudit groupe dithiométhyle.

30. Procédé selon les revendications 25 et 28, dans lequel ledit procédé est efficace pour une utilisation avec une puce de séquençage.

31. Procédé selon la revendication 25, comprenant les étapes :

a) de fourniture d'une matrice d'amorces comprenant une pluralité d'amorces de séquençage ;
b) de mise en contact d'un acide nucléique cible avec ladite matrice d'amorces dans des conditions dans lesquelles lesdites amorces de séquençage s'hybrident audit acide nucléique cible de façon séquence-spécifique, formant de ce fait des complexes cible-amorce entre des parties complémentaires desdites amorces de séquençage et dudit acide nucléique cible ;
c) d'incorporation d'un premier nucléotide modifié par un radical dithiométhyle en position 3' dans au moins une partie d'une amorce de séquençage desdits complexes cible-amorce, ledit premier nucléotide modifié par

un radical dithiométhyle en position 3' étant complémentaire dudit acide nucléique cible ; et
d) de détection de ladite incorporation dudit premier nucléotide modifié par un radical dithiométhyle en position 3', dans laquelle ledit premier nucléotide modifié par un radical dithiométhyle en position 3' est complémentaire de ladite séquence cible au niveau dudit site d'incorporation du premier nucléotide modifié par un radical dithiométhyle en position 3'.

32. Procédé selon la revendication 31, comprenant de plus les étapes :

e) d'élimination dudit groupe dithiométhyle dudit premier nucléotide modifié par un radical dithiométhyle en position 3' incorporé pour former un premier complexe cible allongée-amorce possédant un groupe hydroxyle libre en position 3' ;
f) d'incorporation d'un deuxième nucléotide modifié par un radical dithiométhyle dans ledit premier complexe cible allongée-amorce ; et
g) de détection dudit deuxième nucléotide modifié par un radical dithiométhyle en position 3', dans laquelle ledit deuxième nucléotide modifié par un radical dithiométhyle en position 3' est complémentaire de ladite séquence cible au niveau dudit site d'incorporation du deuxième nucléotide modifié par un radical dithiométhyle en position 3'.

33. Procédé selon les revendications 25 et 31, dans lequel lesdites étapes de détection sont réalisées avant l'élimination dudit groupe dithiométhyle.

34. Procédé selon la revendication 28, dans lequel lesdites étapes de détection sont réalisées après l'élimination dudit groupe dithiométhyle.

35. Procédé selon la revendication 28, dans lequel les étapes a), b), c), d), e), et f) sont réalisées dans des conditions qui ne perturbent pas l'hybridation de ladite amorce audit acide nucléique cible.

36. Procédé selon la revendication 31, dans lequel ledit procédé est efficace pour la préparation d'une pluralité de séquences de nucléotide, lesdites séquences de nucléotide correspondant à des séquences de nucléotide dudit acide nucléique cible recouvrant.

37. Procédé selon la revendication 31, dans lequel ladite étape e) est effectuée dans des conditions qui ne dissocient pas lesdits complexes cible-amorce.

38. Procédé de synthèse d'un oligonucléotide comprenant les étapes :

a) de fourniture d'un premier nucléoside protégé en position 5' lié de manière covalente à un support solide par l'intermédiaire d'une séquence de liaison ;
b) de déprotection dudit premier nucléoside au niveau de sa position 5';
c) de liaison de manière covalente d'un autre nucléoside protégé en position 5' audit premier nucléotide au niveau de la position 5' dudit premier nucléoside ;
d) de déprotection dudit autre nucléoside au niveau de sa position 5'; et
e) de répétition des étapes c) et d) pour additionner des nucléosides protégés supplémentaires, ladite séquence de liaison liant ledit premier nucléotide audit support solide par l'intermédiaire d'un pont dithiométhyle en formant un composé répondant à la formule :

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

dans laquelle

R^1 est choisi dans le groupe consistant en des désoxyribonucléosides, des désoxyribonucléotides, des désoxyribonucléosides ayant une base et/ou un sucre modifiés, et des désoxyribonucléotides ayant une base et/ou un sucre modifiés et R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification.

39. Procédé selon la revendication 38, dans lequel la séquence de liaison est une séquence de liaison contenant un radical dithiométhyle et comprenant, à la suite de l'étape d), la réalisation des étapes suivantes :

e) éventuellement la répétition des étapes c) et d) pour ajouter des nucléosides protégés supplémentaires, produisant de ce fait un oligonucléotide;
f) éventuellement le clivage de manière sélective d'un groupe de protection dudit oligonucléotide, formant de ce fait un oligonucléotide partiellement déprotégé ;
g) le clivage de manière sélective de ladite séquence de liaison contenant un radical dithiométhyle ; et
h) l'isolement dudit oligonucléotide partiellement déprotégé.

40. Procédé selon la revendication 39, dans lequel ledit procédé est efficace pour inverser ledit oligonucléotide, formant de ce fait un oligonucléotide possédant un hydroxy libre en position 3' et étant lié de manière covalente à un support solide.

41. Procédé selon les revendications 38 et 40, dans lequel ledit procédé est optimisé pour une utilisation dans une matrice.

42. Procédé selon la revendication 38, comprenant de plus l'étape de clivage dudit oligonucléotide dudit support solide.

43. Procédé selon la revendication 39, comprenant de plus l'étape de modification de l'extrémité 3' dudit oligonucléotide avec une entité réactive ou détectable.

44. Procédé selon la revendication 39, dans lequel au moins un desdits nucléosides protégés en position 5' comprend une entité dithiométhyle.

45. Procédé de synthèse d'un oligoribonucléotide comprenant les étapes :

a) de fourniture d'un premier ribonucléoside protégé lié de manière covalente à un support solide ;
b) de liaison de manière covalente d'au moins un ribonucléoside modifié par un radical dithiométhyle en position 2' répondant à la formule :

$$R^1\text{-O-CH}_2\text{-S-S-}R^2$$

dans laquelle

R^1 est choisi dans le groupe consistant en des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, et des ribonucléotides ayant une base et/ou un sucre modifiés, et R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification ;

audit premier ribonucléoside pour former un oligoribonucléotide ;
c) de déprotection partielle dudit oligoribonucléotide dans des conditions acides ou basiques ; et
d) de mise en contact dudit oligoribonucléotide avec un agent de réduction dans des conditions neutres, déprotégeant de ce fait ledit ribonucléoside modifié par un radical dithiométhyle en position 2' en ledit oligoribonucléotide.

46. Procédé selon la revendication 45, dans lequel ledit ribonucléoside modifié par un radical dithiométhyle en position 2' est totalement déprotégé en ledit oligoribonucléotide.

47. Procédé selon la revendication 45, dans lequel le pH desdites conditions neutres varie d'environ 5 à environ 9.

48. Procédé selon la revendication 47, dans lequel ledit pH est environ 7.

49. Procédé selon la revendication 45, dans lequel ledit procédé est efficace pour inverser ledit oligoribonucléotide, formant de ce fait un oligoribonucléotide possédant un hydroxy libre en position 3', lié à une phase solide.

50. Procédé selon la revendication 45, dans lequel ledit premier ribonucléoside protégé est lié audit support solide par l'intermédiaire d'un pont dithiométhyle.

51. Utilisation d'un composé modifié par un radical dithiométhyle répondant à la formule :

$$R^1\text{-}O\text{-}CH_2\text{-}S\text{-}S\text{-}R^2$$

ou d'un sel de celui-ci, dans laquelle

R^1 est choisi parmi des acides aminés modifiés ou non modifiés, des peptides, des protéines, des glucides, des stérols, des stéroïdes, des ribonucléosides, des ribonucléotides, des ribonucléosides ayant une base et/ou un sucre modifiés, des ribonucléotides ayant une base et/ou un sucre modifiés, des désoxyribonucléosides, des désoxyribonucléotides, des désoxyribonucléosides ayant une base et/ou un sucre modifiés, et des désoxyribonucléotides ayant une base et/ou un sucre modifiés ; et
R^2 est choisi parmi des hydrocarbures saturés et insaturés, des hydrocarbures aliphatiques à chaîne linéaire ou ramifiée, des hydrocarbures cycliques, des hydrocarbures aromatiques, des hydrocarbures hétérocycliques, des hydrocarbures hétéroaromatiques, et des hydrocarbures substitués tels que des hydrocarbures contenant des hétéroatomes et/ou d'autres groupes fonctionnels de modification, pour le séquençage d'acides nucléiques.